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THE EFFECT OF CHLOROFORM ON THE FACTORS OF COAGULATION

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That chloroform might accelerate the coagulation of blood was suggested to me by Dr. W. W. Palmer. He noticed that blood very rapidly became thickened and lumpy when he attempted to extract substances from it with chloroform.

Howell (1) attempted to use chloroform to isolate prothrombin. In so doing he noticed that a clear oxalated dialyzed plasma of a fasting cat when shaken with chloroform for one to two hours and filtered, clotted. He concludes from this that "prothrombin can be converted to thrombin in a calcium free solution," though in a less effective manner than by calcium salts. In order to investigate further what effect chloroform had on the factors of coagulation, the following observations were made:

To nine tubes each containing five drops[1] of clear oxalated guinea pig plasma, three, five and seven drops[2] of chloroform were each added to three tubes. The tubes were gently shaken to mix the chloroform with the plasma. A firm, very slightly cloudy, non-retractile jelly clot was formed in each tube. The time in which the clot formed varied from 30 to 60 minutes, even in the tubes containing the same amount of chloroform. If fresh blood platelets were added to the plasma chloroform mixture the clot became somewhat retracted.

If from 3 to 7 drops of chloroform were added to the plasma and then an optimum amount of calcium chloride solution 0.5 per cent

[1] 1 drop of plasma measured approximately 0.05 cc.

[2] 1 drop of chloroform measured approximately 0.01 cc.

added immediately or after an interval of 15 to 30 minutes a clot appeared in 6 or 7 minutes. However, when the same amount of calcium chloride but no chloroform was added to the same plasma a clot did not appear until 10 minutes.

This experiment was repeated many times using human, rabbit, dog, cat and guinea pig oxalated plasmas with widely varying results, which did not depend upon the animal from which the plasma was obtained. The oxalated plasmas used had not clotted of their own accord at the end of 48 hours. Sometimes a plasma would clot when chloroform was added to it in as short a time as 20 minutes, again not for 6 to 24 hours and quite as often not at all. A plasma that had been standing over 12 hours seemed to clot upon the addition of chloroform more often than a fresh plasma. Whether 3 or as many as 12 drops of chloroform were added to the 5 drops of plasma seemed to make little difference. Usually if less than 3 drops of chloroform were used there was either no clot or a weaker clot appeared in a longer time.

The clots were usually solid, allowing inversion of the tube, although they sometimes were but sliding jelly. They were, however, not as firm as normal blood clots or clots of oxalated plasma with calcium.

The addition of chloroform to the plasma caused a slightly cloudy opalescence. Sometimes there would also appear a few fine white flocculi, usually only in the tubes with the greater amounts of chloroform. These flocculi would settle with the chloroform to the bottom of the tube and their presence did not affect the clotting time or the nature of the clot.

If the chloroform plasma mixture was shaken very violently it became the consistency of wet sticky snow. This may have been due to a colloidal suspension, for it could be broken up by alcohol.

The effect of larger amounts of chloroform will be spoken of later.

In order that certain plasmas to which chloroform had been added might clot, it was found that the chloroform must be well mixed with the plasma; and also after mixing, the tube must be kept still, because a very little motion delayed markedly the time in which the clot formed as well as its firmness. Even with these precautions, however, many chloroform plasmas failed to clot.

Various oxalated plasmas were dialyzed in celloidin sacs against salt solution to remove the excess of oxalate. Those plasmas which clotted after adding chloroform before dialysis clotted afterwards and those not clotting before did not clot afterwards. Thus variations in the amount of oxalate present was not the cause for the varying action of chloroform.

When an optimum amount of calcium and a few drops of chloroform were added to those plasmas which clotted in the presence of chloroform alone, a clot usually formed in a shorter time than when only calcium was added. Plasmas not clotting upon the addition of chloroform sometimes clotted upon the addition of chloroform and calcium in the same amount of time as which they did with calcium alone. At times a slight acceleration occurred; while occasionally there was a slight delay, usually when the larger amounts of chloroform were added.

In order to decide if prothrombin could be converted to thrombin by chloroform, the following experiment was done and repeated:

FIBRINOGEN[1] DROPS	$CaCl_2$ (0.5 PER CENT) DROPS	CHLOROFORM DROPS	PROTHROMBIN[2] DROPS	REMARKS
10	3			No clot in 24 hours.
10	3		5	Clot in 8 minutes.
10			5	No clot in 24 hours.
10		Amts. varying from 1 to 15 drops.	5	No clot in 24 hours.
10	3	Amts. varying from 1 to 15 drops.	5	Clot in 7–10 minutes.

[1] Pure solution made by modification of Hammarsten's method.

[2] Several solutions made according to Howell's method. American Journal of Physiology, xxxv., 474, 1914.

This experiment shows that chloroform was unable to convert prothrombin to thrombin while calcium did so readily.

Various attempts were made to determine the presence of thrombin and fibrinogen in the serum from the clots formed by adding chloroform to plasma, but neither was found.

The effect of chloroform on thrombin, fibrinogen and antithrombin was next studied.

Chloroform was thoroughly mixed with an equal and with half the amount of a pure solution of thrombin.[3] This caused a slightly opalescent solution but no precipitate. This thrombin acted fully as well as a control specimen at once or after several hours, showing that chloroform did not affect thrombin.

[3] From crystals of thrombin made according to Howell's method. Am. Jour. of Physiol., xxvi, 453, 1910, and xxxii, 264, 1913.

Three to ten drops of chloroform mixed with eight drops of a solution of fibrinogen plasma,[4] or with a solution of pure fibrinogen, and allowed to stand 1 minute to 60 minutes did not weaken their ability to be clotted by thrombin; provided the chloroform did not permit the fibrinogen plasma used to clot within 60 minutes. The pure fibrinogen solution never clotted upon the addition of chloroform.

In fact the chloroform fibrinogen plasma solution clotted with thrombin slightly faster than the control fibrinogen plasma. This may be explained by the effect of the chloroform on the antithrombin (discussed below) contained in the fibrinogen plasma.

That chloroform destroys or renders inactive antithrombin is shown by the following data in Table I obtained from repeated experiments.

TABLE I

THROMBIN DROPS	ANTI-THROMBIN[1] DROPS	CHLOROFORM DROPS	INTERVAL OF TIME (MINUTES) AFTER THROMBIN ADDED BEFORE FIBRINOGEN SOLUTION ADDED	FIBRINOGEN SOLUTION DROPS	CLOT IN MINUTES
2	0	0	0 or 15	8	3
2	0	3 or 5	0 or 15	8	2½
2	1	0	15	8	28
2	1	2, 3 or 6 drops well mixed with antithrombin for 1 min. to 1 hr. before thrombin added.	2, 15 or 30	8	3–5
2	1	Thrombin and antithrombin mixed for 15 min., then 2, 3 or 6 drops added and well mixed.	2 or 15	8	4–7
2	1	3 drops without mixing.	15	8	18
2	1	¼ drop well mixed.	15	8	15
2	1	10 drops not mixed.	15	8	5

[1] For discussion of antithrombin test see paper by Minot and Denny in print for Archives of Int. Med.

It was found that in order to have the chloroform render the antithrombin ineffective the two must be well mixed.

Ether was found to act similarly to chloroform. Like chloroform its presence sometimes allowed an oxalated plasma to clot and was

[4] From dried specimens of plasma made according to Howell's method. Archives of Int. Med., xiii, 76, 1914.

able to render ineffective antithrombin. In contrast to chloroform, however, the few observations made showed that ether always delayed the clotting of an oxalated plasma with calcium, causing often only a weak clot to form, and unlike chloroform it would slightly weaken the power of a solution of thrombin.

Carbon bi-sulphide, petroleum ether, benzol, xylol, potassium chloride, magnesium sulphate, calcium chloride and weak alkalies were found to have no inhibiting power on the action of antithrombin. Dilute weak acid, however, did inhibit the action of antithrombin, as has also been pointed out by Collingwood and MacMahon (2).

Doyon (3) and also Billard (4) have been able to isolate from the liver and other organs by a chloroform extraction process an antithrombin thought to be of a nucleo-protein nature. It is probable that this substance is not the same as Howell's antithrombin of the circulating blood, for Doyon is able to carry it through certain procedures such as heating to high temperatures that would destroy Howell's antithrombin.

Jobling and Petersen (5) have shown that chloroform and ether may remove the antitryptic power of serum, which is probably due to compounds of unsaturated fatty acids. They were able to recover the antitrypsin from the extracts by saponification. It takes several days' extraction at room temperature to remove completely the antitryptic power of serum while antithrombic power is removed at once.

Attempts were made to see if Howell's antithrombin could be recovered from chloroform extracts of the blood, for it was thought that it might be similar to Doyon's antithrombin or to antitrypsin. To a solution of antithrombin (oxalated plasma heated to 60°C. and filtered) varying amounts up to twice as much chloroform were added and the mixture centrifuged. The opalescent solution free from chloroform in the upper half of the tube exhibited very feeble antithrombic power as compared to the control. The material at the bottom of the tube with the chloroform, after removal of the chloroform exhibited no antithrombic power, while Doyon's extracts of antithrombin from organs did. Other attempts with serum and with plasma unheated and heated to 60°C. have been unsuccessful so far in recovering Howell's antithrombin from chloroform or ether extracts. However, a further study especially on the effect of complete saponification of such extracts is necessary before we can say whether Howell's antithrombin like antitrypsin is absorbed by chloroform and ether or whether it is destroyed. Serum antitrypsin can be absorbed by starch.

From my observations various experiments have shown that starch has no effect on antithrombin.

Zak (6) has shown that petroleum ether extracts of the red blood corpuscles contain a substance which retards coagulation. I have been unable to obtain with chloroform extracts of the red cells any substance with antithrombic power.

Large amounts of chloroform precipitated fibrinogen and prothrombin from oxalated plasma, but no material was obtained with antithrombic power. An equal amount or more of chloroform added to oxalated plasma, well shaken and centrifuged, caused a thick creamy white precipitate to form as a band in the middle of the tube. This precipitate was soluble in normal salt solution. It clotted with calcium and also thrombin, showing that it contained prothrombin and fibrinogen. No antithrombin could be demonstrated in a solution of this precipitate. In one instance a solution of this precipitate clotted by itself after 18 hours as a weak, water-clear clot, showing that some thrombin was present. In three other instances no clot occurred in 24 hours. In the centrifuge tube above the precipitate there remained a clear solution (A) equal in volume to the faintly cloudy solution (B) below the precipitate. Neither A nor B had any antithrombic power.

Small amounts of prothrombin and fibrinogen were usually found in A, rarely in B.

Equal amounts of ether and plasma acted similarly to chloroform and plasma, although ether caused a smaller precipitate.

Whether a trace of free thrombin exists in the circulating blood is not known, but we do know that thrombin begins to be formed from prothrombin as soon as the blood is shed. Hence the speed and method with which blood is collected may be factors in determining how much free thrombin exists in an oxalated plasma.

The blood from which the clear plasma for the above experiments was obtained was drawn from the heart or big veins of animals and veins of humans with an all glass syringe previously sterilized and rinsed in salt solution. The blood was at once mixed with an oxalate solution (0.1 per cent sodium oxalate in 0.9 per cent salt solution). In some instances the oxalate was placed in the barrel of the sryinge so that the blood became mixed with the oxalate as it was drawn. With such procedures, more especially the former, it is probable that a trace of free thrombin will occur in the plasma.

Finding that antithrombin was rendered inactive by chloroform it seemed that perhaps the reason why some oxalated plasmas clotted

upon the addition of chloroform was that some contained more free thrombin than others; so that after the chloroform had rendered inactive the antithrombin, the free thrombin could clot the fibrinogen. This would explain the results obtained in the above experiments.

In order to test this point, the following procedures were undertaken: Blood was collected with a paraffined cannula from the carotid artery of 4 cats and 2 rabbits and run directly into oxalate solution. The fresh clear plasma from these animals did not clot upon the addition of chloroform. Of five plasmas similarly obtained from veins one clotted feebly upon adding chloroform. Clear plasma obtained from blood, which had been allowed to remain a few minutes in a glass syringe before being mixed with oxalate solution, did not clot by itself. Such plasma did, however, almost always clot when chloroform was added to it.

Therefore it seems that the reason that chloroform permitted some oxalated plasmas to clot, which did not clot themselves, is that it renders inactive the antithrombin and allows any free thrombin to clot the fibrinogen.

From this it would appear that the addition of thromboplastic substances which neutralize antithrombin ought to clot similar oxalated plasmas in a similar manner as chloroform. It was found that a solution of kephalin[5] (the active thromboplastic material) in sufficient amount did.

A rabbit with typical acute chloroform poisoning from 1 cc. of chloroform in oil given subcutaneously 48 hours before showed a diminished amount of antithrombin. As the total amount of chloroform injected was small and as chloroform is broken up in the body, it does not seem likely that the low antithrombin content was due to the action of the chloroform on the circulating antithrombin. The liver is important in the formation of antithrombin and Denny and Minot (7) have shown that in dogs with liver destruction from phosphorus poisoning the antithrombin in the blood may be markedly diminished. Though chloroform produces destruction of a different part of the liver than phosphorus perhaps the decreased antithrombin in the rabbit was due or partially due to destruction of the liver tissue. Acidosis may perhaps be an added cause. Acidosis[6] occurred in the chloroform poisoned rabbit and it not infrequently occurs in phosphorus

[5] Prepared by Howell's method. Am. Jour. of Physiol., xxxi, 1, 1912.

[6] Tested by method described by Rowntree, Marriott and Levy. Transactions of 13th Annual Meeting of the Association of American Physicians, 1915.

poisoning[7] and has been found to occur in other experimental and some clinical conditions where the antithrombin was low. The effect of acidosis on antithrombin will be discussed in another paper.

SUMMARY

1. Antithrombin is rendered inactive by chloroform and ether, thus allowing free thrombin if present in an oxalated plasma to clot fibrinogen.

2. Prothrombin is not converted to thrombin by chloroform.

3. Chloroform can precipitate both fibrinogen and prothrombin from an oxalated plasma.

4. Chloroform does not weaken the action of a solution of pure thrombin. Ether does slightly.

5. Antithrombin could not be recovered from chloroform or ether extracts of serum or plasma, unheated or heated to 60°C., and is not exactly identical to antitrypsin or to Doyon's antithrombin.

6. In one chloroform poisoned rabbit the antithrombin of the blood was decreased below normal.

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[7] L. G. Rowntree. Personal communication.

PERISTALSIS AND COORDINATION IN THE EARTHWORM

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It is our purpose to analyze the normal progressive movements of the earthworm (Lumbricus terrestrialis) with a view to determining the physiological factors involved.

According to the descriptions of Friedländer (1) and of Biedermann (2) the progressive movements of this animal are brought about by a passive elongation proceeding from the anterior end. This is succeeded by a forcible extension which travels posteriorly. The third and last phase is an active shortening due to contraction of the longitudinal muscles. The peristaltic wave travels backward and as a result the animal moves forward since the setae are directed backward and, in the contracted portion, act as hold-fasts.

The origin of the peristaltic waves which cause the earthworm to move forward, has been ascribed by Friedländer to traction; Biedermann agrees with Friedländer and maintains that the initiation of peristalsis is due to a stretching of the myodermal sheath, and not to contact with a rough surface, for the same regular peristalsis may occur in a worm suspended in air. That it was the tension on the myodermal sheath with which these authors were concerned, and that the pull on the nerve cord was not responsible for the initiation of peristalsis, we have determined by dissecting the nerve cord free from surrounding tissues and applying varying degrees of traction to it alone; no peristalsis resulted; but immediately the animal was pulled by the body wall sufficiently to move it over the surface on which it rested, peristalsis began.

While it is clear that tension on the myodermal sheath plays an important rôle in the initiation of peristalsis, the underlying mechanism

[1] We wish to acknowledge our indebtedness to the management of the Marine Biological Laboratory at Woods Hole for providing facilities for carrying on this work.

by which tension produces such a result, is not clear, and it is certain that former experimenters have overstepped the mark in excluding all effects of surface stimulation. It therefore has seemed to us that even tension effects might be further analyzed on the basis of sensory impulses.

To test the reasonableness of this suggestion, we obtained graphic records of the effects of mechanical stimulation in the following way. A worm preparation was clamped near the posterior end and the anterior upper end was attached to a recording lever.[2] In order to exclude the possibility of any tension effects, the stimuli were applied below the clamp. If such a preparation be stimulated, when quiescent, by touching with a moist camel's hair brush, peristaltic waves as a rule start.

In different tests it was found that groups of peristaltic waves could be started by tapping. This result frequently succeeded only when several such stimuli were applied at regular intervals (fig. 1, 12, 10, 8). If instead of being quiescent, the preparation showed regular peristalsis, repeated tapping causes a marked acceleration, as noted in figure 1-x, in which case the rate of rhythm increased from six to twelve per minute. These results show that our assumption was correct and that even a regular peristaltic rhythm may be initiated by sensory stimulation. This led to an attempt further to analyze the effects of sensory stimulation.

THE EFFECTS OF SENSORY STIMULATION

If a worm preparation or an intact worm be sharply touched at any point the result is an immediate shortening throughout the entire length of the worm; a reaction which in no way simulates any phase of peristalsis. A light touch or a gentle pinch elicits a response of an entirely different nature; applied to the anterior end, in addition to the localized shortening, an active lengthening takes place and the animal moves away from the point of stimulation. This extension may involve the entire animal. If, however, the stimulus is applied to the posterior end there is a shortening throughout the entire extent of the preparation, somewhat more marked at the point of stimulation.

[2] In this work, unless otherwise specified, a 'worm preparation' was made by cutting off both the anterior and posterior ends of an individual so as to provide a middle portion of suitable length. In clamping, a modified Gaskell clamp was used and the preparation sufficiently compressed to hold the part firmly without impairing the conductivity of the nerve cord.

The animal is pulled forward, since the setae act as hold-fasts, being extruded coincidentally with the contraction of the longitudinal muscles.

It is evident that the direction of propagation of the impulse determines the nature of the response, whether it be a lengthening or a shortening, and that stimulation somewhere in the mid-course of the preparation should determine different events anterior and posterior to the point of stimulation.

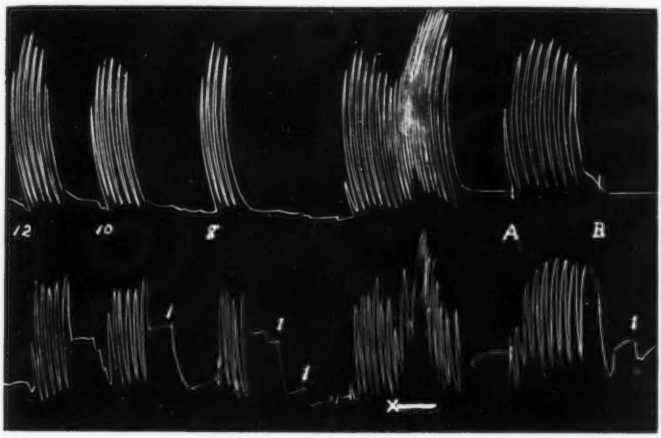

Fig. 1. Upper tracing of anterior half and lower tracing of posterior half of a worm preparation lightly clamped near the mid point. At each of the points marked 1 a single touch stimulus was applied just above the clamp and caused elongation of the posterior half. The figures 12, 10 and 8 represent number of contact stimuli applied to the posterior half near the clamp at the rate of about one per second. The group of contractions above x was spontaneous and the rhythm was accelerated by repeatedly touching the posterior piece for one minute, as indicated by the horizontal line. At *A* the anterior half was stroked beginning at its anterior end. At *B* same half was stroked from the clamp back to the anterior end.

It was a simple matter to devise a method of obtaining a graphic record and analyzing in detail these phenomena of shortening and lengthening. The worm preparation was firmly clamped in the middle, as described above, without impairing the conductivity of the ventral nerve cord. The free ends were attached to levers recording simultaneously on a smoked drum. When a light mechanical stimulus was applied immediately above the clamp a marked shortening of the whole

anterior portion took place and was attended by a simultaneous lengthening of the whole posterior portion below the clamp (fig. 1.) This usually started a peristaltic wave which began at the anterior end of the preparation and which traveled through and involved the posterior part. It would seem then that a peristaltic wave may begin with a shortening and does not necessarily involve a preliminary extension of the initiating part (Biedermann, loc. cit.) Weak faradic stimulation applied to a point on the surface of the animal gave the same result as mechanical stimulation. In other words, local stimulation gives a picture which is identical with peristalsis as seen in the intestine of higher animals, viz., contraction above and relaxation below the point of stimulation.

These results explain an observation which we have made on the effects of stroking the surface of a suspended preparation with a moist brush. If the strokes be from the anterior to the posterior end, peristaltic waves are set up due to the fact that there is a shortening anterior to the point stimulated (fig. 1, *A*). If the direction of the stroke is reversed, and is made from the posterior towards the anterior end of the preparation, peristalsis is inhibited and the animal remains extended (fig. 1, *B*). Sometimes by repeated upward stroking complete quiescence in the extended state could be obtained, at other times the duration of quiescence corresponded to that of several peristaltic waves. We also found that the normal rate of peristalsis is always accelerated by downward stroking with a camel's hair brush; for example, in one preparation with the lower end weighted, giving 10 to 12 peristaltic waves per minute, the rate was increased to 16 by stroking downward. In the same preparation peristalsis was completely inhibited for 30 seconds by upward stroking, and when the weight was removed upward stroking caused extension with complete quiescence.

The results of this experiment may be applied to the explanation of the coordinated creeping movements of a worm preparation when drawn over moist filter paper, anterior end foremost (Friedländer) (3). In terms of sensory stimulation of the worm surface, the forward pulling is equivalent to backward stroking, and therefore sets up a series of peristaltic waves. If now, the worm preparation be drawn backward posterior end foremost over damp filter paper, shortening and peristalsis is inhibited, the piece simply extends and if care be exercised to avoid acute stimulation or overextension the piece may remain quietly extended for a long time. This effect is readily understood, for backward pulling over a rough surface produces a sequence of sensory effects

similar to those due to upward stroking with a brush. From these results it follows that when a worm creeps forward each peristaltic wave will tend to involve the whole worm and will be followed by another; thus progression continues until some other opposing factor, such for example as central fatigue, is interjected. If on the other hand a backward movement should take place, the sequence of the stimuli tends to bring the animal into a state of rest which will persist until the animal is again definitely excited to peristaltic activity.

Moreover, the fact that peristaltic waves may be set going by causing shortening of several segments, as is the case in a downward stroke in which more and more of the worm is anterior to the point of stimulation, would again indicate that peristalsis can be initiated by a contraction of the longitudinal muscles. Such a conclusion is further sustained by the fact that upward stroking, in which more and more of the worm lies posterior to the point of stimulation and therefore extends, inhibits peristalsis and may leave the worm extended and quiescent. Clearly extension does not necessarily initiate peristalsis.

In view of these facts it seems reasonable to suppose that the effects of traction in bringing about peristalsis in a preparation hanging free in the air are to be accounted for on the assumption that the stretching of the skin causes sensory impulses to be set up. These impulses cause reflex shortening anteriorly and lengthening posteriorly. This would also account for the fact that when a worm preparation is hung up by either the anterior or posterior end, the peristaltic wave with rare exceptions begins at the anterior end.

Since sensory stimuli (contact, or traction), acting upon the earthworm tend to set up peristaltic movements, we may, with right, raise the question whether all normal peristaltic movements may not be the result of sensory stimuli.

THE RÔLE OF THE VENTRAL NERVE CORD

The differences in the effects produced anterior and posterior to the point of stimulation, still further resolves itself into a question whether they be due to differences in the character of the stimuli or to the intermediation of the central nervous system. In order to put this question to the test we have studied the effects of stimulation of the nerve cord itself. After anaesthetizing a worm preparation in a solution of ether in water, portions of the nerve cord at anterior and posterior ends was dissected free from surrounding tissue. The responses of these portions of nerve cord to selected faradic stimulation are identical with those

elicited by mechanical cutaneous stimulation described above, but the responses may vary with the strength and duration of the stimulation as noted in the following experiments.

Two centimeters of the dorsal myodermal sheath and intestine were removed near the middle of an anaesthetized worm preparation. The nerve cord was exposed and carefully separated from the ventral portion of the body wall. The latter strip was left connecting anterior and posterior sections of the worm, and was made fast to a cork plate by four pins. The nerve cord was kept moist with Ringer's solution, and was known to be uninjured by the fact that, after recovery from the anaesthetic, peristaltic movements in the posterior piece of the worm

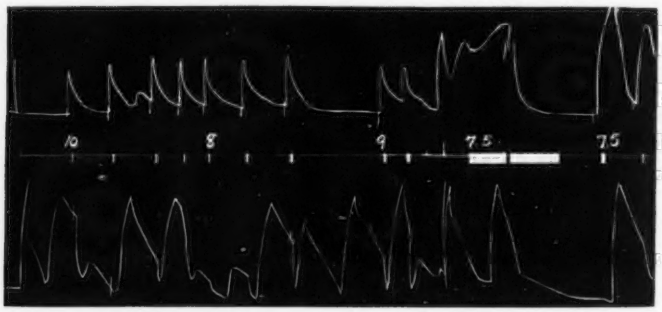

Fig. 2. Effects of faradic stimulation of the nerve cord in the middle of the worm preparation. The upper tracing records events of the anterior half and lower tracing of the posterior half of the preparation. The stimulation periods are recorded on the middle line. The figures represent the strength of the stimuli in terms of centimeters to which the secondary coil has been withdrawn from the primary.

followed in regular sequence those which began in the anterior segments. In this case the portion of the ventral musculature did not conduct the impulses for the contraction wave did not extend through it and the impulses were still conducted when the cut extended through this connection but left the nerve cord intact. On other preparations the ventral bridge of muscle and skin was left intact but the coordination of the posterior with the anterior portions of the preparation ceased immediately upon cutting the ventral nerve cord. We thus effectually and conclusively proved the correctness of Biedermann's contention that the peristaltic impulses are conducted by the nerve cord.

If a small cork plate were used in pinning the above preparation it could be clamped to a stand, and graphic records of the contractions of the anterior and posterior ends of the worm could be easily obtained by attaching them to separate levers. By means of a thread loop the isolated section of the nerve cord was slightly raised and brought to rest upon a pair of platinum electrodes which were carefully kept from contact with any other tissue. Faradic shocks were obtained by the use of a Porter inductorium. If the nerve cord was stimulated with appropriate shocks (with the coil at 10 to 8 cm., fig. 2) the anterior section shortened,[3] whereas, the posterior lengthened at first and subsequently shortened in response to the impulse propagated back from the anterior half. Shocks continued for two minutes with the coil at 7.5 cm. produced a tonic contraction of the anterior part and a prolonged lengthening of the posterior part (fig. 2). Stronger but shorter stimulation resulted in some cases in rapid rhythmical peristalsis of the posterior part and persistent contraction of the anterior part of the preparation. The experiment proves that sensory stimuli, do not determine the character of the muscular response by virtue of their nature, but that the responses are determined by the direction the impulses travel in the nerve cord, and ultimately by the connection and the orientation of the elements in the central nervous system. These findings harmonize the coordination of the movements of the earthworm with the analyses made for crustacea by Loeb and Maxwell (5) and for the vertebrates by Loeb and Garrey (6).

While the responses described above harmonize perfectly with those obtained from mechanical cutaneous stimulation, and can be elicited at will by selecting the proper strength of faradic stimulation of the nerve cord, they are not by any means invariable, for changing the strength of the stimuli may change the reaction; nevertheless, one can always predict the character of the response. In a quiescent aboral preparation the nerve cord at its anterior end was stimulated. With the secondary coil at its maximum distance from the primary and inclined at an angle of 45°, stimulation for one second caused only a slight localized twitch. At the angle 30°, half of the preparation nearest the electrodes twitched, while at 15° the whole preparation gave a twitch followed by a peristaltic wave. Increasing the duration of the weaker stimulation caused the initial twitch, persistent shortening of the more anterior segments, and a peristaltic rhythm. With the

[3] A. J. Carlson (4) observed shortening anterior to point of stimulation in Bispira.

secondary coil parallel to and 12.5 cm. from the primary, the whole preparation remained shortened during the period of stimulation—a result which can be due only to a preponderant contraction of the longitudinal musculature. With still stronger stimuli (10 cm.) there was invariably a writhing and squirming of the preparation, sometimes followed by peristalsis. Faradization stronger than 7.5 cm. caused a striking reversal in the nature of the reaction: all writhing and peristalsis ceased and the preparation became quiet in extreme extensions.[4]

The squirming was due to combined irregular contraction of both circular and longitudinal muscular coats; while the extension was due to relaxation of the longitudinal and contraction of the circular coat. Although the absolute strength at which these different results were obtained varied with individual preparations with recovery from anaesthesia and with fatigue due to stimulation of the nerve cord, the relative effects of weak, moderate and strong stimulation could always be elicited.

MUSCULAR PHENOMENA

So far we have referred to the phases of peristalsis only as a lengthening and shortening. Obviously the shortening may be due to a contraction of the longitudinal muscles, and the lengthening either to a relaxation of all of the musculature or to a contraction of the ring muscles accompanied by a relaxation of the longitudinal ones.

The action of the circular musculature can easily and clearly be demonstrated if the myodermal sheath be slit along the median dorsal line. In an otherwise normal worm in which a 3 or 4 cm. slit of this sort had been made, an observation of the progressive movements showed the elongation phase of peristalsis to be due to a double process, viz. the relaxation of the powerful longitudinal musculature and the contraction of the weaker ring muscles of the corresponding segments. In consequence the dorsal slit widened in spite of the decreased diameter of the animal in this region. The relaxation of the longitudinal muscles accompanying the contraction of the circular set is a beautiful and striking example of 'contrary' or 'reciprocal' innervation. The succeeding shortening of the segments is due to a contraction of

[4] Stimulation of the nerve cord was never seen to produce squirming of the portion anterior to the point of stimulation. This, with the added fact that strong shocks produce quiescence and extension while more moderate stimulation produces violent squirming excludes these movements from the criteria of pain and confirms the analysis of pain sensations made by Norman (7).

the longitudinal musculature accompanied by a relaxation of the ring muscles of the segments involved. The relaxation phenomenon is clearly shown by the closure of the slit in spite of the increased diameter of the segments in question. Here again we have 'contrary' or 'reciprocal' innervation in the opposite sense to that considered above.

These phenomena have been duplicated for the greater part by electrical stimulation of the nerve cord exposed in the fashion already described. Stimulation (coil at 9 cm.) gave a slow simultaneous even widening of the slit for a considerable distance posterior to the point of stimulation, with no contraction of the longitudinal muscles. The result was therefore a lengthening of the posterior part. At the same time the portion anterior to the exposed nerve shortened because of contraction of the longitudinal muscles but at the same time showed contraction of the circular ones, a difference from the normal which is probably due to the strength of stimuli used.

PROPAGATION OF STIMULI AND FATIGUE

No account of the normal reactions of the worm would be compete without an added reference to the propagation of stimuli of different strengths and to the fatigue phenomena which develop with repeated reaction to stimuli.

Some effects of varying the strength of stimulation of the nerve cord have been noted in a previous section; to these may be added those which are constantly obtained in experiments of the following type. In one preparation, faradic stimulation for one second with the coil at 7.5 cm. caused only 25 segments to respond; at 4 cm. a response was elicited from 50 segments while at 0, 90 segments, i.e., the whole worm preparation, contracted. It is clear from results such as these that the intensity of the response decreases with distance from the point of stimulation, and the strongest response occurs at that point. The relation of this fact to the local contraction which always occurs as the result of mechanical or electrical stimulation is obvious. Stimulation, either mechanical or faradic, of the cutaneous surface gave similar results, the proximate segments being more intensely affected. No attempt was made to eliminate direct stimulation of the musculature.

After the nerve cord had been stimulated repeatedly with faradic shocks of a given strength, the muscles ceased to respond. With an increase in strength of the stimulus there was a renewal of the muscular response. Since cutaneous stimulation gave a local response, the rise

of threshold in this experiment indicated central nerve fatigue. Furthermore, a stimulus of moderate intensity causes a muscular response of a certain number of segments, which, after continued stimulation, no longer responded to this stimulus. When the intensity of the stimulus was slightly increased only a limited number of proximate segments showed contraction of the musculature. The failure of the stronger stimuli to involve the musculature of the more distant segments, which had at no time shown contraction, again proves that the ventral cord had been fatigued. Reference has already been made to the possible relation of this fatigue to the suppression of progressive movements of the animal.

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THE CARDIO-INHIBITORY CENTER

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The cardio-inhibitory center in the medulla oblongata has not yet been definitely localised. Laborde (1), who attempted to do so, employed the method of puncture (piqûre) and obtained inhibition from a point situated, according to his drawing, in the lateral part of the fourth ventricle some distance cephalad to the dorsal vagus nucleus. He appears to have believed that the cardio-inhibitory fibers originate in the nucleus ambiguus, which he refers to by the designation "noyaux accessoires de l'hypoglosse et des nerfs mixtes (pneumogastrique, spinal, glossopharyngien)." His observations were made on cats and dogs.

Schaternikoff and Friedenthal (2), in a study directed to ascertain in which medullary rootlets the cardio-inhibitory fibers emerge, applied unipolar faradisation with a needle electrode to the medulla. They made use of curarised rabbits. They did not determine the location of the inhibitory center and indeed obtained no cardiac slowing by stimulation of the surface of the fourth ventricle but only after plunging the electrode into the deeper layers of the medulla.

The histological researches of Kohnstamm (3) and the more extensive researches of van Gehuchten and Molhant (4) led these writers to infer that the cardio-inhibitory fibers arise in the dorsal vagus nucleus. Since this conclusion is at variance with the results of excitation referred to above we undertook the following experiments in the hope of deciding the question.

METHODS

In our experiments, which were performed on the dog, the animal was anaesthetised first with chloroform and ether and the narcosis was maintained by an intravenous injection of chloralose (0.2 per cent solution).

A median incision was made at the back of the neck and the muscles were detached from the skull and reflected so as to expose the occipital bone and the occipito-atloid ligament. The occipital bone was cut away with bone forceps almost, but not quite, as far forward as the transverse sinus, bleeding being checked by the use of wax. The caudal portion of the cerebellar vermis was then carefully removed so as to bring to view the floor of the fourth ventricle in the vicinity of the dorsal vagus nucleus. The ventricular floor thus revealed lies at the bottom of a deep well of structures and in order to obtain a satisfactory illumination of it an electric head-light was found necessary.

The spinal cord was divided at the level of the atlas vertebra, the lamina of this being first cut away. The object of the procedure was to avoid the rise in blood-pressure which otherwise takes place on faradisation of the medulla. Following the section artificial respiration was at once instituted.

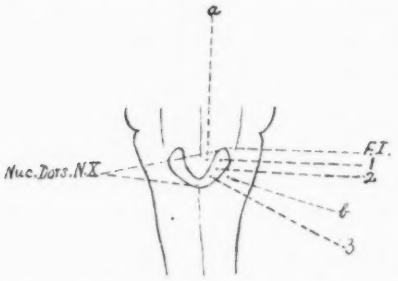

Fig. 1. Diagram of medulla oblongata of dog. Natural size. *F.I.*, inferior fovea; *Nuc.Dors. N.X.*, dorsal vagus nucleus; *a*, *b*, *1*, *2*, *3*, points at which faradisation was applied.

Unlike Schaternikoff and Friedenthal (2) we did not employ curare, owing to the fact that it raises the threshold of the cardio-inhibitory mechanism, a circumstance which is disadvantageous when accurate localisation is to be made. The drug, indeed, was not necessary because, with the weak currents used, no muscular movements were detectable beyond a very slight trembling of the head; movements of the trunk and limbs were, of course, precluded by the division of the cord. The currents used by Schaternikoff and Friedenthal must have been very strong and, as the cord was not cut, curare was necessary.

Faradisation was applied by the unipolar method, the stimulating electrode being of the Sherrington type for cortical localisation; the indifferent electrode was secured to a hind-limb. In order to obtain satisfactory responses we found it essential to keep the medulla warm by the application of hot, moist pads.

The blood-pressure in the carotid artery was recorded by a Hürthle manometer. The animal was secured with the abdomen resting on the table, the head being firmly clamped.

EXPERIMENTAL RESULTS

On examining the floor of the fourth ventricle the dorsal vagus nucleus (ala cinerea, trigonum vagi) is easily discernible by its grey, translucent appearance (fig. 1).

It is an elongated structure about 6 mm. in length, wider in front and gradually tapering backwards to meet its fellow of the opposite side. At its cephalic extremity there is a small depression, the inferior fovea.

The procedure in localising the effects in the nucleus was as follows: While the blood-pressure was being recorded stimulation was applied

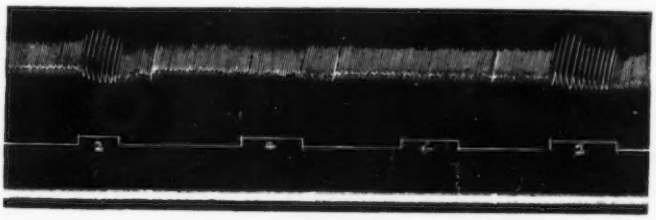

Fig. 2. Record of cardiac effects on stimulating points on medulla oblongata shown in figure 1. Sec. dist. 10 cm. Time in 1/5 secs.

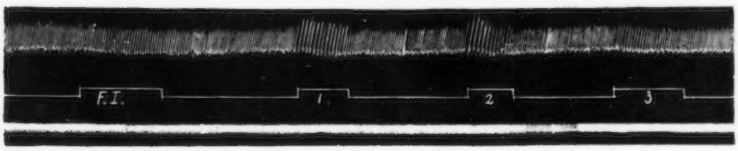

Fig. 3. Record of cardiac effects on stimulating points on medulla oblongata shown in figure 1. Sec. dist. 10 cm. Time in 1/5 secs.

to the nucleus with a current intensity sufficient to evoke a definite cardio-inhibitory effect. The current was then reduced as much as it was possible to do and still obtain slight though definite inhibition from the nucleus. With this minimal current the surface of the ventricle in the neighborhood of the nucleus was explored. No inhibition was evoked mesially or laterally to the nucleus, even at points a millimetre or less from that; but upon returning to the nucleus inhibition was at once elicited. These facts are illustrated in figure 2, in which it may be seen that no effects were obtained from stimulating points *a* and *b* beside the nucleus but a distinct one from point *2* on the nucleus itself. These points are shown on the diagram of the medulla.

Having localised cardiac inhibition in the nucleus this was next interrogated from end to end with the electrode. The entire nucleus was observed to yield inhibition. Inhibition could also be evoked from the inferior fovea, although the response was invariably less pronounced than that from the nucleus itself. It is probable that the inhibition obtained from the inferior fovea is produced in part reflexly by stimulation of the sensory fibers of the tractus solitarius accessible at this point to the current. Stimulation of the inferior fovea was always accompanied by swallowing movements (5). The caudal part of the nucleus sometimes gave a weaker inhibitory effect than the cephalic part but, as this difference was not invariable, we do not attach much significance thereto. The effects of stimulation of the inferior fovea and various points on the nucleus are shown in figure 3. The points referred to in the record are indicated on the drawing of the medulla; they were selected at random and others in their neighborhood yielded similar results.

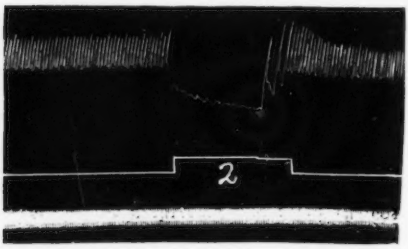

Fig. 4. Record of cardiac effect on stimulating point on medulla oblongata shown in figure 1. Sec. dist. 9 cm. Time in 1/5 secs.

With currents slightly stronger than those used for localisation complete inhibition can be elicited from the dorsal vagus nucleus. This is shown in figure 4 in which stoppage of the heart occurred on stimulation of the middle point of the nucleus.

Laborde (1) claimed to have excited inhibition by puncture of the medulla at a point considerably in front of the dorsal vagus nucleus; but he does not appear to have attempted any very definite localisation and, in fact, such would be impossible with his procedure. His impression that the cardio-inhibitory center is in the nucleus ambiguus is contrary to the results obtained by histologists and by us.

Schaternikoff and Friedenthal (2) asserted that they were unable to excite inhibition when the electrode was applied to the surface of the fourth ventricle and only succeeded in doing so after thrusting it into the substance of the medulla. They also stated that inhibition was readily evoked from the terminations of the posterior columns.

We have found, on the contrary, that when the medulla is exposed carefully it is always possible to obtain inhibition from the surface of the dorsal vagus nucleus with weaker currents than from the posterior columns. Schaternikoff and Friedenthal make no mention of the currents they employed but presumably they were strong since with weak ones they could scarcely have got inhibition from the interior of the medulla. Their failure to evoke inhibition from the floor of the fourth ventricle was probably due to injury in the preparation or to their not having kept the medulla warm. The latter point we found to be most important since, if the medulla becomes cooled, the response is lost.

SUMMARY

The results of our experiments show that the cardio-inhibitory center is situated in the dorsal vagus nucleus and are thus in harmony with the histological investigations of Kohnstamm and of van Gehuchten and Molhant.

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THE EFFECTS OF AQUEOUS EXTRACTS OF ORGANS UPON THE CONTRACTIONS OF UNSTRIATED MUSCLE FIBERS

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Our first communication dealt with an aqueous extract of the thyroid gland. We tested the effects of the different substances which can be separated from it upon the heart action, respiration and blood pressure in dogs, and recorded the results in the usual kymograph tracings. We found that the nucleo-proteins, globulins and coagulable proteins, when administered intravenously in dogs, showed no demonstrable activity. But the filtrate which remains after the removal of these substances exhibited great activity. It produced a noticeable and immediate fall in blood pressure, together with a slight coincident deepening of respiration. But it did not produce any appreciable acceleration of the heart's action. This filtrate was divided into two portions by evaporating to dryness and taking up with 95 per cent alcohol. This gave "alcohol soluble" and "alcohol insoluble" portions. The former was found to manifest the same activity in approximately the same proportion as the original first filtrate which remains after the removal of the nucleo-proteins, globulins and coagulable proteins.

A further separation was made by adding basic lead acetate to the "alcohol soluble" portion until no further precipitation occurred. This yielded what we have termed our "lead precipitate" and "lead filtrate" portions. The "lead filtrate" proved to be the active portion and the richer in iodin. We have retained and used the first filtrate (which remains after the removal of the nucleo-proteins, globulins and coagulable proteins) under the designation of the "thyroid residue."

All of the parts of an aqueous extract of the thyroid which we have tested contain iodin in different amounts. An extract of the pig's thyroid is much richer in iodin than that of the sheep. When the ni-

trogen content of a dose of pig thyroid is made the same as that of a sheep residue, the sheep residue shows less activity in the kymograph tracings than the pig. But when the iodin content of the dose of each residue is made the same, the effect of each upon blood pressure are approximately the same. Hence, the vaso-dilating principle which is present only in the "residue" portion of an aqueous extract of the thyroid is not contained in an active form in all of its iodized proteins, but it exists in some particular, iodized molecule. This is to be sought in the "alcohol soluble" part and the "lead filtrate" from the original entire residue.

Our second communication dealt with the similar substances which can be isolated from aqueous extracts of the pituitary, parathyroid, thymus and adrenal glands and from the pancreas, liver and muscle. We found that the residue portion of these extracts, or the filtrate which remains after the removal of the nucleo-proteins, globulins and coagulable proteins, was the only portion which showed activity in the kymograph tracings. Each "residue," with the single exception of that of the adrenal gland, exhibited a peculiar and definite vaso-dilating power which, when the dose of residue was standardized by its nitrogen content, seemed to be characteristic of the organ from which the residue was derived. The residue from the whole adrenal gland showed a marked vaso-constrictor effect, but the kymograph tracing shows quite a different curve from that produced by the usual 1-1000 solution of commercial adrenalin. From these investigations it has seemed reasonable to conclude that every organ produces some active substance which can be found only in the residue of the organ from which it is derived, and is "active" in the sense that it produces through the circulation some demonstrable physiological change in one or more other organs.

The present communication is an attempt to confirm the location of the active principle of every organ in the residue part of its aqueous extract, and at the same time to ascertain something of its physiology. The simplest and most direct experiments are those with smooth muscle fiber.

A small strip of this tissue was removed from different portions of the intestine and from the uterus of a freshly killed cat, and suspended from the short arm of a writing lever, in a cylinder containing 275 cc. of Locke's solution. This preparation was maintained at 38°C., by immersion in a vessel of water to which a small flame could be constantly applied. A large flask containing the Locke's solution, which

was constantly kept at 38°C., was arranged to syphon into the cylinder holding the suspended muscle segment, in order that the muscle could be washed and the cylinder refilled with warm fresh solution as often as might be required. By this method the dilutions of the different substances whose effects were to be tested could be calculated in terms of nitrogen computed as protein.

The fractions of the aqueous extracts which we have tested consist of the nucleo-proteins, glogulins, coagulable proteins and the filtrate or residue which remains after their removal. The extracts of the pituitary and adrenal glands, like those of the others, were each made from the entire organ. The "alcohol soluble" portion of the thyroid residue in the previous kymograph tracings seemed to contain *all* of the active material which exists in the original residue, but in these muscle tracings was not as active as the *whole* thyroid residue.

TEST SUBSTANCE	AMOUNT OF NITROGEN COMPUTED AS PROTEIN MGS. PER CC. SOL.	IODIN MGS. PER CC. SOL.
Thyroid residue	26.15	0.2
Alcohol soluble part	16.8	0.2
Alcohol insoluble part	27.	0.1
Thyroid globulin	24.	0.025
Thyroid coagulated protein	8.4	0.01
Thyroid nucleo-protein	6.1	0.0275
Thymus residue	41.3	0
Thymus globulin	1.1	0
Thymus nucleo-protein	12.7	0
Spleen residue	43.25	0
Spleen globulin	2.5	0
Pituitary residue	24.5	0
Pancreas residue	112.7	0
Pancreas nucleo-protein	2.5	0
Liver residue	45.	0
Alcohol soluble part	42.5	0
Liver nucleo-protein	7.2	0
Adrenal residue	22.6	0
Adrenal nucleo-protein	4.2	0
Adrenal globulin	3.5	0
Parathyroid residue	16	0

The amount of the substance which was tested was computed by the nitrogen content in terms of protein, and was thus made the same in each instance. A dose of 5 minims of thyroid residue which contained

8.2 mgs. of protein was taken as the base or standard. This, when mixed with the 275 cc. of Locke's solution in the cylinder holding the unstriated muscle segments, amounted to a dilution of approximately 1 in 10,000.

Effects upon the contractions of the cat's uterus
(Average results from many experiments.)

SUBSTANCE TESTED	REACTION	SYMBOL
Thyroid residue	Very strong	(++++)
Alcohol soluble part	Perceptible	(+)
Alcohol insoluble part	Doubtful	(±)
Thyroid globulin	None	(0)
Thyroid coagulated proteins	None	(0)
Thyroid nucleo-proteins	Doubtful	(±)
Thymus residue	Perceptible	(+)
Thymus globulin	None	(0)
Thymus nucleo-protein	None	(0)
Spleen residue	Good	(++)
Spleen globulin	None	(0)
Pituitary residue	Very strong	(++++)
Pituitrin (Burroughs Wellcome)	Very strong	(++++)
Pancreas residue	Perceptible	(+)
Pancreas nucleo-protein	Doubtful	(±)
Liver residue	Good	(++)
Alcohol soluble part	Perceptible	(+)
Liver nucleo-protein	Doubtful	(±)
Parathyroid residue	Very strong	(++++)
Adrenal residue	Perceptible	(+)
Adrenal nucleo-protein	None	(0)
Adrenal globulin	None	(0)

Of the large number of tracings required to establish these findings, there are submitted for inspection only two which illustrate the average of the results in the cases of the residues of the thyroid (Fig. 1), and pituitary glands (Fig. 2). The addition of a few drops of 1: 1000 adrenalin chloride solution causes an immediate paralysis of the previously induced and more or less characteristic residue contractions. This paralyzing effect of epinephrin upon the uterus is well recognized. The adrenal residue contains much epinephrin but does not like adrenalin paralyze the contractions induced by other residues, except in the case of the pituitary residue. The characteristic contractions induced by the pituitary residue are paralyzed by the adrenal residue as they are also by adrenalin chloride.

In all of these experiments contractions were produced mainly by the residue portion of the extracts. Those which resulted from the addition to the Locke's solution of thyroid or of parathyroid residues

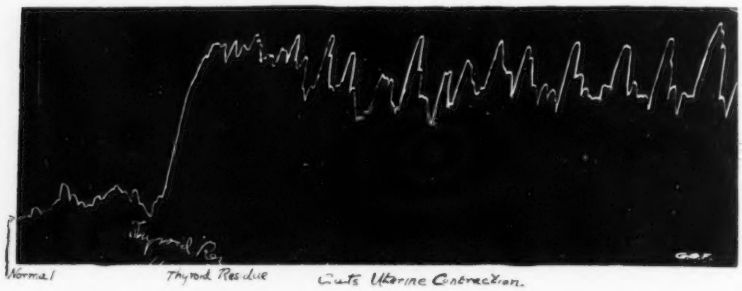

Fig. 1. Thyroid residue. Cat's uterine contraction.

were of longer duration than those from the pituitary residue. With the parathyroid residue the muscle reaction consists of regular, vigorous contractions followed by partial relaxations. The contractions are at first of the clonic type, but soon pass into the tonic type. The ef-

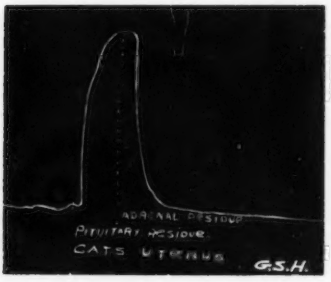

Fig. 2. Effect of pituitary residue on cat's uterus followed by adrenal residue.

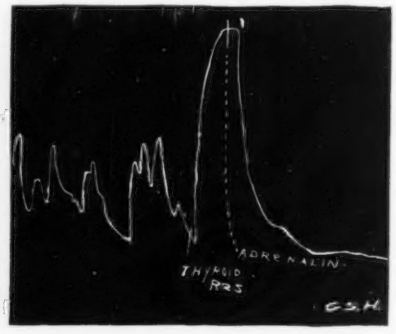

Fig. 3. Effect of thyroid residue on cat's ileum followed by adrenalin chloride.

fects of the thyroid residue are about as pronounced as those from the parathyroid residue, but differ in that fibrillary twitchings are superimposed on the vigorous contractions, and later there is a less marked type of tonic contractions.

With the same method and the same dosage we next tested the effects of the different fractions of aqueous extracts of organs upon the contractions of the muscle segments taken from the duodenum, ileum and the proximal and distal portions of the colon.

The results have been recorded in the following table:

7

SUBSTANCE TESTED	DUODENUM	REACTION UPON MUSCLE FORM		DISTAL PT.
		Ileum	Colon Proximal pt.	
Thyroid residue	Strong (+++)	Very strong (++++)	Perceptible (+)	None (0)
Alcohol soluble part	Strong (+++)	Perceptible (+)	None (0)	None (0)
Alcohol insoluble part	Perceptible (+)	None (0)	None (0)	None (0)
Thyroid globulin	None (0)	None (0)	None (0)	None (0)
Thyroid nucleo-protein	Perceptible (+)	Perceptible (+)	None (0)	None (0)
Thyroid coagulated protein	None (0)	None (0)	None (0)	None (0)
Parathyroid residue	Very strong (++++)	Very strong (++++)	Good (++)	Strong (+++)
Liver residue	Perceptible (+)	Good (++)	None (0)	Perceptible (+)
Alcohol soluble part	Perceptible (+)	Strong (+++)	Perceptible (+)	Good (++)
Liver nucleo-protein	None (0)	None (0)	None (0)	None (0)
Spleen residue	Doubtful (±)	Perceptible (+)	Perceptible (+)	Doubtful (±)
Spleen nucleo-protein	None (0)	None (0)	None (0)	None (0)
Spleen globulin	None (0)	None (0)	None (0)	None (0)
Thymus residue	Doubtful (±)	Very strong (++++)	Perceptible (+)	Doubtful (±)
Thymus globulin	None (0)	Doubtful (±)	None (0)	None (0)
Thymus nucleo-protein	None (0)	Perceptible (+)	None (0)	None (0)
Pancreas residue	Perceptible (+)	Strong (+++)	Perceptible (+)	Perceptible (+)
Pancreas nucleo-protein	None (0)	None (0)	None (0)	None (0)
Adrenal residue	Perceptible (+)	Good (++)	Perceptible (+)	Perceptible (+)
Pituitary residue	Strong (+++)	Strong (+++)	Strong (+++)	Strong (+++)

After the contraction induced by any residue has become manifest, the addition of adrenalin produces an immediate relaxation. This is shown in Figs. 3, 4 and 5.

From these findings it appears that the globulins and coagulable proteins of all organs show no effect upon the contractions of smooth muscle fiber from any part of the gut. The nucleo-proteins rarely gave a perceptible or doubtful reaction which might indicate a content of some proenzyme or incompletely formed active principle.

The thyroid residue does not act on all parts of the intestine with equal force. It produces a strong reaction upon the duodenum and a very strong reaction upon the ileum. But upon the proximal portion of the colon its reaction is slight and upon the distal part none.

The parathyroid residue produces a very strong reaction upon the duodenum and ileum, but only a good reaction upon the proximal part of the colon, while upon the distal portion its reaction is very strong.

The pituitary residue seems to produce the same vigorous reaction upon all parts of the small and large intestine.

The thyroid, parathyroid and pituitary all have a similar pronounced effect upon both uterine and intestinal muscle. The pancreas produces a strong reaction upon the ileum, but only a perceptible reaction upon the remainder of the intestine and the uterus.

The adrenal residue shows effects which are very similar to those of the pancreas.

The thymus residue produced a very strong reaction upon the ileum, but only a very slight or doubtful reaction upon the colon and the duodenum. The residues of the spleen, and liver were only mild stimulants to intestinal contractions.

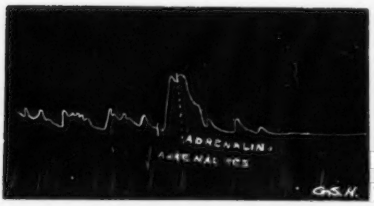

Fig. 4. Effect of adrenal residue on cat's ileum followed by adrenalin chloride.

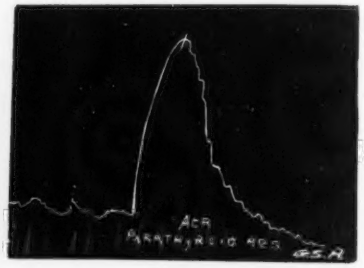

Fig. 5. Effect of parathyroid residue on cat's ileum followed by adrenalin chloride.

CONCLUSIONS

(The term "residue" refers to that portion of an aqueous extract of an organ which remains after the removal of the nucleo-proteins, globulins and coagulable proteins.)

1. The residues of an aqueous extract of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands, and from the liver, pancreas and spleen, contain most, if not all, of the internal secretions of these organs.

2. Each residue produces a characteristic stimulating effect upon the unstriated muscle fibers of the cat's uterus. This stimulation is paralyzed by adrenalin.

3. The residue of each organ acts differently upon different portions of the unstriated muscle fibers of the cat's intestine. When the residue

stimulates the contraction, the addition of adrenalin produces an immediate paralysis.

4. Adrenalin is generally accepted as acting upon the intermediate substance between the end plates of the terminal filaments of the sympathetic and unstriated muscle. Therefore, these residues of organs must act upon some portion of the termination of the sympathetic nerves, and each residue produces its effect by a different chemical or physico-chemical action.

THE FEEDING OF YOUNG CHICKS ON GRAIN MIXTURES OF HIGH AND LOW LYSINE CONTENT

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(From the Kentucky Agricultural Experiment Station)

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It has been shown by Osborne and Mendel (1) in an exhaustive series of experiments on the feeding of albino rats, that lysine is primarily responsible for the stimulation of growth. In this connection E. H. Nollau (2) and, independently, Grindley, Joseph and Slater (3) have recently made quantitative determinations of the amino-acids contained in various commercial feeding stuffs and other sources of protein by the Van Slyke (4) method, with the view of determining the lysine content of such mixtures. In this connection, it also occurred to one of us, Kastle, that it would be of interest to conduct a series of feeding experiments on young chicks, using grain mixtures containing a low and high lysine content respectively. With this end in view, two lots of young chicks of mixed breed, each lot containing ten chicks and selected entirely at random from the entire lot, were chosen for the experiment. These lots are herein designated as Lot I and Lot II, respectively. During this feeding experiment, which lasted over a period of eight weeks, viz, from May 13 to July 6, 1915, these chicks were kept under strictly comparable conditions, so far as their general habits of life were concerned. The experiments were carried on in a basement room of the Experiment Station building, floored with concrete, the temperature of which ranged from 23.5°C. to 27°C., and which during a part of the day was exposed to sunlight from basement windows having a southeasterly exposure. The two lots of chicks were separately confined in wire runs, $4\frac{1}{2}$ by 4 feet, the bottom of the run being floored with common lumber and covered over with a layer of earth and straw. All of the chicks were freely supplied with water, gravel, charcoal and oyster shells during the progress of the experiment and

[1] The feeding experiments described in this paper were carried on entirely by Messrs. Buckner and Nollau.

about once a week there was placed in each run a piece of grass sod from one to two feet square. All of the chicks were supplied at intervals with sprouted oats, and each run was provided with a hover of the usual type in which the chicks were placed at night.

At the beginning of the experiment the weights of the two lots of chicks were as follows: Lot I (10 chicks) 438 grams, and Lot II (9 chicks) 338 grams, or an average weight per chick, in Lot I, of 43.8 grams, and in Lot II, of 43.1 grams. The chicks of Lot I received a mash twice a day, morning and evening, consisting of equal parts by weight of finely ground wheat, wheat bran, sunflower seed and hemp seed, moistened with skimmed milk, and once a day at noon, they were given a coarsely ground grain mixture of wheat, hemp seed and cracked corn.

On hydrolysis the mash fed to the chicks of Lot I gave the following numbers for amino-acids, by the Van Slyke method:

Ammonia N	12.09
Melanin N	7.42
Cystine N	2.09
Arginine N	17.48
Histidine N	3.28
Lysine N	3.80
Amino N filtrate	38.53
Non-amino N filtrate	13.94
al	98.63

Tow ls the end of the experiment the sprouted oats furnished these c icks was replaced by cabbage. The mash, exclusive of the milk, contained 18.09 per cent of protein, and the grain mixture 16.29 per cent protein.

The grain mixture fed to Lot I gave, on hydrolysis, the following numbers for amino-acids:

Ammonia N	11.66
Melanin N	10.50
Cystine N	2.72
Arginine N	11.36
Histidine N	6.21
Lysine N	2.23
Amino N filtrate	41.89
Non-amino N filtrate	15.67
Total	102.24

The mash was prepared with a small amount of sour skimmed milk varying between 15 and 20 cc. In this connection it should be borne in mind that owing to the habits of the chicken it is practically impossible to feed any given lot of chickens precisely the same amount of food at any one time, or rather, it is impossible to determine, except in a general way, whether they have eaten all the food presented to them at any one time. This difficulty was overcome as completely as possible in this work by feeding each lot of chickens approximately the amount of food that they would clean up at any particular time, these several amounts being accurately weighed at every feeding. For example, in the morning, at eight o'clock, Lot I received 30 grams of the mash, and at noon they received from 15 to 25 grams of the grain mixture. Then at 5 p.m. they received a second 30-gram portion of the mash. In the same way Lot II received 30 grams of a mash consisting of finely ground barley, rice, hominy and oats, 100 grams each, and 56 grams of gluten flour. This mash contained 18.1 per cent of protein, and gave the following numbers for amino-acids on hydrolysis:

Ammonia N	17.85
Melanin N	7.22
Cystine N	4.29
Arginine N	5.93
Histidine N	7.38
Lysine N	.50
Amino N filtrate	42.99
Non-amino N filtrate	15.68
Total	101.84

At noon the chicks of Lot II received from 30 to 50 grams of a grain mixture consisting of equal parts of barley, rice and hominy, which gave the following numbers for amino-acids on hydrolysis:

Ammonia N	12.68
Melanin N	9.40
Cystine N	1.95
Arginine N	6.77
Histidine N	7.76
Lysine N	.79
Amino N filtrate	40.08
Non-amino N filtrate	20.25
Total	99.68

This grain mixture was found to contain 8.74 per cent of protein and hence twice as much of it was fed to the chicks of Lot II as of the grain

mixture fed to Lot I. The mash supplied to the chicks of Lot II was prepared with protein-free milk.[2] The two lots of chickens were weighed at the beginning of the feeding experiments, at noon on May 13, and again at intervals shown in the following tables:

TABLE I—LOT I

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF EACH CHICK	AVERAGE INCREASE IN WEIGHT IN GRAMS	PERCENTAGE AVERAGE INCREASE
1915					
May 13.....	10	438	43.8		
May 19.....	9	486	54.0	10.2	23.30
May 25.....	7	540	77.1	23.1	42.77
June 1.....	7	802	114.6	37.1	47.82
June 8.....	7	1113	159.0	44.4	38.74
June 15.....	7	1517	216.7	57.7	36.30
June 23.....	5	1650	330.0	113.3	52.20
June 29.....	5	2063	412.6	82.6	25.00
July 6.....	5	2553	510.6	98.0	23.75

TABLE II—LOT II

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF EACH CHICK	AVERAGE INCREASE IN WEIGHT IN GRAMS	PERCENTAGE AVERAGE INCREASE
1915					
May 13.....	9	388	43.1		
May 19.....	9	450	50.0	6.9	16.00
May 25.....	8	477	59.6	9.6	19.20
June 1.....	8	588	73.5	13.9	23.32
June 8.....	8	707	88.3	14.8	20.10
June 15.....	8	848	106.0	17.7	20.00
June 23.....	7	928	132.5	26.5	25.00
June 29.....	7	1086	155.1	22.6	17.00
July 6.....	7	1195	170.7	15.6	10.00

It will be seen from the above results that 5 chickens of Lot I at the conclusion of the experiment weighed 2553 grams, whereas 7 chickens

[2] The protein-free milk used in these experiments was prepared according to the method described by Osborne and Mendel (Carnegie Pub.) except that the casein was precipitated with lactic acid and the product neutralized with lime, our reason for making this change in the original directions of Osborne and Mendel being to eliminate common salt from the ration, which is said to be toxic to the chicken.

of Lot II weighed 1195 grams, or 5 average chickens of Lot II would have weighed 853.5 grams. The striking difference in these two lots of chicks is further shown by a comparison of the photographs of the two lots made on July 5. In this connection it should be borne in mind that the focal distance was the same in both instances so that the photographic comparisons are exact. One of the photographs shows the largest and best developed cockerel of each of the two lots. It will be observed that there are marked differences in the feathering of the two lots of chickens, Lot I showing the feathering characteristic of the mature chicken, whereas Lot II still showed the feathering of the young and immature chick at the conclusion of the experiment. Great difference in the two lots of chickens was also shown in their general activity during the progress of the experiment, the chickens of Lot I being greatly more active than the chickens of Lot II. It was also observed that the chickens of Lot II consumed more charcoal than the chickens of Lot I. No diarrhea or other evidences of digestive disturbances were observed except in the case of one chicken of Lot I and this was only noticeable one day. Five chickens of Lot I and three of Lot II died during the early progress of the experiment, one of the chicks of Lot II dying before the feeding experiment was actually begun.[3] During the progress of the experiment both lots of chickens were abundantly supplied with tap water to which a small amount of lime was added each day.

It is well known that certain of the materials used in the make up of the ration of Lot I have been used to advantage in feeding fowls and birds. For example, the value of sunflower seed and skimmed milk as a feed for chickens is well recognized by poultrymen and hemp seed always forms a large part of the ration of small birds in captivity, such as the canary. The desire shown by the young chick for hemp seed is remarkable. It has been observed that out of a grain mixture containing this material, they will pick out every hemp seed before eating the remainder of the ration, and in this connection it is of interest to note in passing that of all of the substances used in our feeding experiments, hemp seed is richest in lysine.

[3] The high mortality shown by these two lots of chicks is inexplicable for the reason that they received all possible attention and were kept under sanitary surroundings. In this connection it should be borne in mind, however, that chickens hatched late in the spring show an abnormally high mortality as compared with chickens hatched very early in the spring. This is probably due to loss of virility on the part of the cock fertilizing the eggs.

At the conclusion of the feeding period on July 6, the chickens of Lot II were put on the rations fed to Lot I. On July 13, the chickens of Lot II were found to weigh 1539 grams, showing a total gain of 344 grams for the lot, or an average weight per chick of 219.9 grams, as compared with 170.7 grams per chick on July 6, or an increase in 7 days of 49.2 grams per chick as compared with an average gain per week of 15.9 grams during the regular period of the experiment.[4]

The chicks used in these experiments were hatched under the hen on May 9. They were brought to the Experiment Station on the 11th and the feeding experiment was begun on May 13. In the interval between May 11 and May 13 all of the chickens received small amounts of Purina chick feed containing 11 per cent protein.

It is evident from these results that whereas the chickens of Lot I grew normally, the chickens of Lot II were undoubtedly stunted in their growth. This difference in the nutrition of these two lots of chicks is due, in all probability, to the difference in the amount of lysine received by the two lots and possibly to a difference in the quantity and nature of the fats contained in the two rations. Thus the mash fed to the chicks of Lot I contained 13.08 per cent of fat, and the dry grain mixture 8.21 per cent, whereas the mash fed to the chicks of Lot II contained only 1.8 per cent of fat, and the grain mixture 1.0 per cent. It should be borne in mind in this connection, that Osborne and Mendel (5) and also McCollum (6) and his associates have shown that certain of the natural fats contain substances which stimulate animal growth. Such fats are known to be present in butter, cod liver oil, the yolk of the egg, corn meal (7), etc., and such growth-promoting fats are doubtless contained in many other grains and vegetable prod-

[4] This, in itself, is a striking confirmation of the fact that the ration fed to the chickens of Lot I is greatly in excess of the ration fed originally to the chicks of Lot II in its power to stimulate growth. This rapid increase in weight shown by the chicks of Lot II during the time that they were on the ration of Lot I, indicates that while their growth was stunted on the first ration, they still possessed the power to grow rapidly on the ration of Lot I. In this connection it may be said that during the long period in which the chicks of Lot II were on the original ration, they were not only stunted in growth but very timid, especially for hand raised chicks, whereas during the shorter period of one week, during which time they were on the ration of Lot I, they became much less nervous and excitable and very gentle. Another striking difference between the chicks of Lots I and II is that shown in the combs of the cockerels of the two lots, the combs of the cockerels of Lot I being red and well developed, whereas the combs of the cockerels of Lot II were pale and undeveloped. This is clearly shown in photograph 5.

ucts. Such being the case we would be unwarranted in attributing the greatly increased growth of the chicks of Lot I as compared with the chicks of Lot II, entirely to differences in the character of the proteins furnished the two lots of chicks. The difference shown by the two lots, however, is striking and unmistakable. It, therefore, remains to be determined whether this difference is due to differences in the protein or fats, one or both.

In order, therefore, to throw further light upon this point, two lots of pure bred white Leghorn chicks, Nos. III and IV, were fed upon the same rations that were fed to Lots I and II except that to the ration supplied to Lot IV there was added sufficient butter fat to bring the fat content up to that of the ration fed to Lot I; our reason for using butter fat in this connection being that McCollum and Mendel have shown this to be a growth-promoting fat.[5]

The feeding experiments on Lots III and IV were carried out in the following manner: out of the same lot of pure bred white Leghorn chicks, twelve chicks were selected at random from the entire lot to compose Lot III, the remaining twelve composing Lot IV. Lot III was found to weigh 488 grams or an average of 40.6 grams per chick. Lot IV was found to weigh 471 grams, or an average of 39.25 grams per chick.

Lot III was fed on mash No. 1 morning and evening, and on grain mixture No. 1 at noon. Lot IV was fed on mash No. 2 morning and evening and on grain mixture No. 2 at noon, the mash No. 2 and grain mixture No. 2 containing butter fat. Both of these lots received shredded cabbage, which they ate with avidity, and in both runs a square foot of fresh sod was placed every other day. The chicks of both lots also had free access to charcoal, gravel, oyster shells and water to which a small amount of lime was added daily and in which at intervals was placed a small amount of permanganate.

The results of these feeding experiments are given in Tables III and IV.

It will be seen from Table III that five of the chicks died during the first three weeks of the feeding period.

[5] Obviously the best method of settling this point would have been to add to the ration fed to the chicks of Lot II the fat extracted from the ration fed to Lot I in the quantities contained in the ration of Lot I, viz.: 13.08 per cent in the mash and 8.21 per cent in the grain mixture. Experimental difficulties were encountered, however, in the effort to extract the fat contained in the rations fed to Lot I.

TABLE III—LOT III

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE INCREASE IN GRAMS	AVERAGE PERCENTAGE INCREASE	GRAMS FEED PER CHICK PER DAY
1915						
July 1	12	488	40.6			5.3
July 9	9	487	53.8	13.2	32.5	10.0
July 15	9	607	67.4	13.64	25.3	10.0
July 22	7	625	89.4	22.0	32.5	12.8
July 29	7	945	135.0	45.6	51.0	15.0
August 5	7	1315	185.0	50.0	37.0	18.0
August 12	7	1657	236.7	51.7	27.9	21.0
August 19	7	2000	285.7	49.0	20.7	24.0
August 26	7	2510	358.5	72.8	25.4	27.0
August 29	7	2769	395.6	36.8	10.3	27.0
September 4	*7*	*2886*	*412.3*	*17.0*	*4.3*	*27.0*
September 11	*7*	*2988*	*426.8*	*14.5*	*3.5*	*27.0*
September 18	*7*	*3200*	*459.1*	*32.3*	*7.5*	*27.0*

TABLE IV—LOT IV

DATE	NO. OF CHICKS	TOTAL WEIGHT OF CHICKS	AVERAGE WEIGHT OF CHICKS	AVERAGE INCREASE IN GRAMS	AVERAGE PERCENTAGE INCREASE	GRAMS FEED PER CHICK PER DAY
1915						
July 1	12	471	39.25			5.3
July 9	10	461	46.1	6.85	17.4	10.0
July 15	10	517	51.7	5.6	12.1	10.0
July 22	6	322	54.5	2.8	5.4	12.8
July 29	6	398	66.3	11.8	21.65	15.0
August 5	6	462	77.0	10.7	16.1	18.0
August 12	6	515	85.8	8.8	11.4	21.0
August 19	6	592	98.6	12.8	14.9	24.0
August 26	6	660	110.0	11.4	11.5	27.0
August 29	6	708	118.0	8.0	7.3	27.0
September 4	*5*	*863*	*144.0*	*26.0*	*22.1*	*27.0*
September 11	*5*	*931*	*186.2*	*42.2*	*29.3*	*27.0*
September 18	*5*	*1178*	*235.6*	*41.4*	*26.5*	*27.0*

At the beginning of this feeding experiment, July 1, seven average chicks of Lot III weighed 284.2 grams or an average of 40.6 grams. At the end of the feeding experiment, on August 29, the 7 chickens weighed 2769 grams, or an average of 395.6 grams per chick, or within a period of 59 days each chick, of Lot III gave an average gain of 355.

grams. On the other hand it will be seen from Table IV that 6 chicks of the lot died within the first three weeks of the feeding experiment. At the beginning of the feeding experiment, six average chicks of this lot weighed 235.5 grams, or an average of 39.25 grams per chick. At the end of the feeding period, on August 29, these 6 chicks weighed 708 grams, or an average of 118 grams per chick. In other words the chicks of Lot III showed an average gain per chick of 277.6 grams over the chicks of Lot IV or a percentage gain of 235.

The weekly gains of these two lots of chicks are shown in Tables III and IV. The differences shown by these two lots of chicks, at the end of the feeding period, were very striking. The chickens of Lot III were strong, growthy and perfectly feathered, in contrast to the chicks of Lot IV which although in perfect health, were markedly stunted in their growth and showed the feathering characteristic of a much younger chick. All of the chicks of Lot IV, for example, at the end of the feeding period, still showed the yellow color and appearance of the newly hatched chick about the head and neck. The external sexual characteristics of these two lots of chicks showed also the most striking differences. In Lot III the cockerels, for example, were easily distinguished from the hens and both showed well developed, highly colored gills and combs, whereas the chicks of Lot IV showed no well developed external sexual characteristics whatever, it being impossible to distinguish between the cockerels and the hens, the combs of both being rudimentary and colorless. These differences are shown to some extent in the photographs (8 to 10 inclusive) of these two lots of chickens, which were made on August 27. On the other hand, nothing except a colored photograph could bring out fully the striking differences shown by these two lots of chicks.

On August 29 the rations fed to these two lots of chicks were reversed, with the most striking result, as also shown in Tables III and IV, the numbers being given in italics. It will be seen that the average percentage gains of Lot III are 5.1 against 25.9 for Lot IV for the three weeks. Within one week after reversing the rations fed to Lots III and IV the external sexual characteristics of the chicks of Lot IV became noticeable and at the end of three weeks were very pronounced. The yellowish down about the heads of the chicks of Lot IV had disappeared and the feathering is becoming that of the normal fowl of this age.

It is evident from these results that the marked differences shown by these two lots of chicks in rate of growth and development cannot

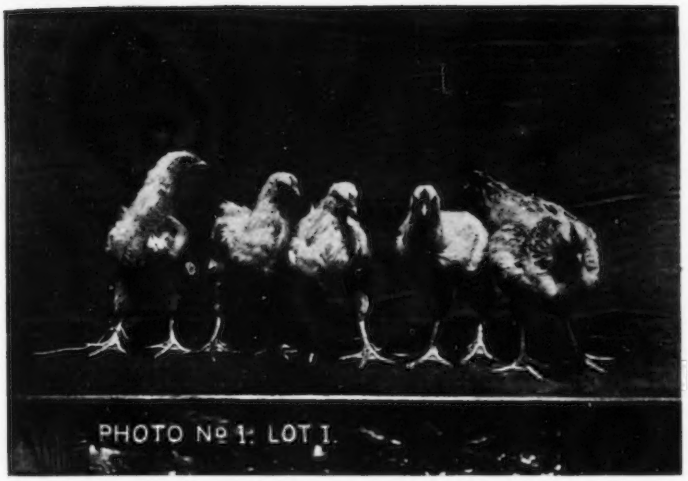

PHOTO Nº 1. LOT I.

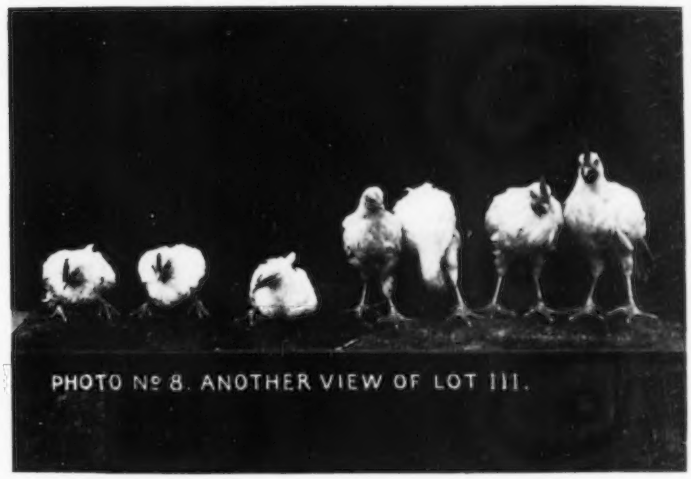

PHOTO Nº 8. ANOTHER VIEW OF LOT III.

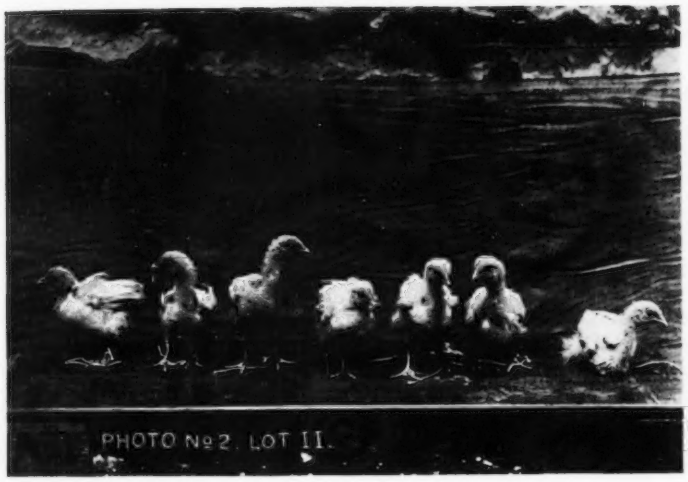

PHOTO Nº 2. LOT II.

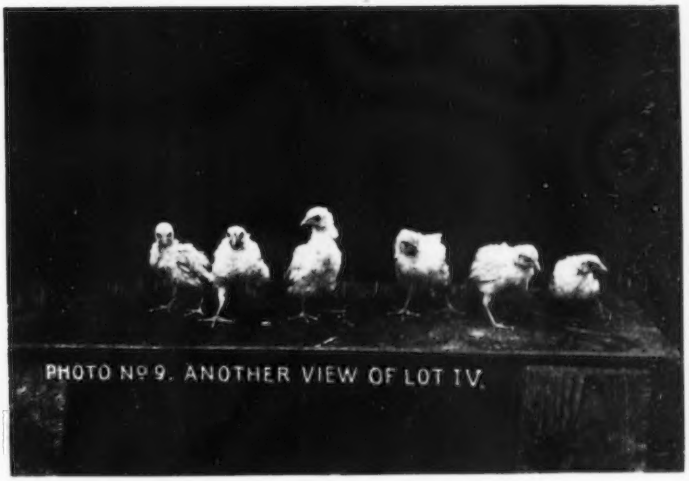

PHOTO Nº 9. ANOTHER VIEW OF LOT IV.

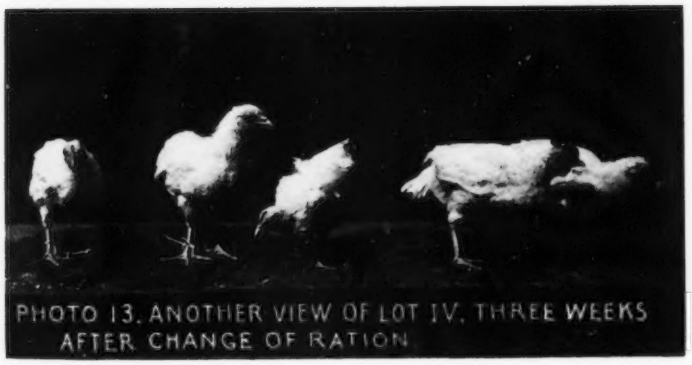

PHOTO 13. ANOTHER VIEW OF LOT IV. THREE WEEKS AFTER CHANGE OF RATION

PHOTO 14. THE TWO BEST COCKERELS IN LOTS III AND IV, RESPECTIVELY.

PHOTO №5. COCKERELS FROM LOT I. AND LOT II.

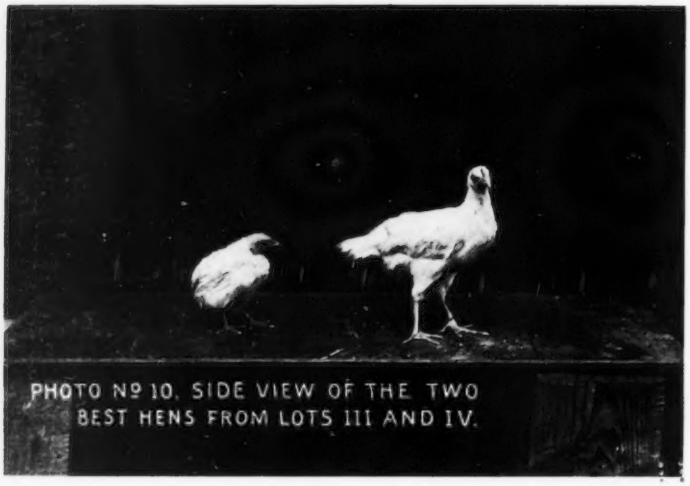

PHOTO № 10. SIDE VIEW OF THE TWO BEST HENS FROM LOTS III AND IV.

In the legends, wherever the words "another view" occur, it signifies that several photographs were made of these two lots of chicks. To save space, however, it has been found necessary to reduce the number of illustrations and hence these particular photographs have been selected.

be ascribed to the fat content of the two rations, but rather to differences in the amino-acid content of the two rations and in all probability to differences in the lysine content.

As indicated in the above, we have not as yet been able to satisfactorily extract the fats and oils contained in mash and grain mixtures No. 1. We are still hopeful of accomplishing this, however, in order to determine the effect of adding the fats and oils contained in ration No. 1 to ration No. 2.

In conclusion, we desire to point out the value of feeding experiments with the chicken both from a practical and scientific standpoint. The financial returns to poultrymen from a successful method of feeding young chicks and laying hens would be enormous. From a purely scientific standpoint, the young chick lends itself well to feeding studies, for the reason that it reaches maturity in from nine months to one year, depending on the breed. For these reasons, it is proposed to continue these investigations as rapidly as time will permit.

(1) Osborne and Mendel: Journ. Biol. Chem., 1914, xvii, 325; Zeit. f. Physiol. Chem., 1912, lxxx, 307; Carnegie Inst. of Washington, Pub. No. 156, pts. 1 and 2.
(2) Nollau: Jour. Biol. Chem., 1915, xxi, 611.
(3) Grindley, Joseph and Slater: Jour. Amer. Chem. Soc., 1915, xxxvii, 1778.
(4) Van Slyke: Jour. Biol. Chem., 1911, x, 15.
(5) Osborne and Mendel: Jour. Biol. Chem., 1913, xvi, 423; 1914, xvii, 401.
(6) McCollum: Jour. Biol. Chem., 1913, xv, 167.
(7) McCollum: Jour. Biol. Chem., 1915, xxi, 179.

ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

II. The Correlation Between Strength of Stimuli and the Direct and Reflex Nerve Response

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CONTENTS

INTRODUCTION

In a preceding paper[1] we have described the flexion-reflex in the decerebrate cat as recorded electrically in a flexor muscle and its motor nerve. In the present paper we propose to report certain observations on the gradation in intensity of the stimuli employed during these experiments, and to discuss the bearing of these observations on the spread of reflexes and the doctrine of "graded synaptic resistance." We have observed the reflex responses to a series of single induction

[1] Forbes and Gregg: This Journal, 1915, xxxvii, 118.

shocks graded as to intensity, and subsequently recorded from the afferent nerve used to induce the reflex, monophasic action currents corresponding to a similar series of stimuli applied in the same way.

The subject of the correlation of the intensity of muscular response with the intensity of reflex stimulus has been summarized by Sherrington.[2] He states that the "correspondence between the intensity of external stimulus and reflex end-effect" is less close than that found in comparing the stimulus applied to a motor nerve with either the contraction of the innervated muscle or the electrical response of the nerve itself. He cites Biedermann as finding an "all-or-none" relation in the reflex response of a cooled frog to single induction shocks. But he shows clearly that "graded intensity of reflex-effect does occur." He demonstrates this fact specifically in the spinal mammal in the case of the flexion-reflex (which has been the subject of our experiments), the scratch-reflex and the crossed extension reflex; although he finds an approximately "all-or-none" relation in the "extensor-thrust." In the gradation of response prolongation is even more marked than increase in intensity. It should be noted that in all Sherrington's cases the stimuli were not single but repeated, and applied to the skin and not to an afferent nerve trunk. The importance of these facts will be considered later.

In a recent paper,[3] published since the completion of our experiments, Sherrington and Sowton report that with single induction shocks of graded intensity applied to an afferent nerve trunk, a more extensive gradation of flexor reflex contraction is found than in the case of the same muscle stimulated directly through its motor nerve. They show that grading in the reflex persists through a range of stimuli subsequently shown to be supramaximal for the nerve-muscle preparation. Their records are all myographic and they furnish no direct evidence as to gradation of activity in the stimulated afferent nerve trunk itself.

In regard to the spread of reflexes resulting from an increase in the intensity of stimulation, Sherrington wrote as follows:[4] "If by appropriate stimulation of the skin of the foot . . . . the ordinary flexion-reflex of the hind limb of the dog be evoked, the extent of the reflex increases with increase in the intensity of the stimulus. The re-

[2] Sherrington: Integrative Action of the Nervous System, 1906, 70–72.

[3] Sherrington and Sowton: Journ. Physiol., 1915, xlix, 331. Cf. also Graham Brown: Proc. Roy. Soc. B., lxxxvii, 132. The latter showed extensive grading of reflex contraction in tenuissimus muscle.

[4] Sherrington: Op. cit., 150.

flex-effect spreads over a larger and larger field, irradiating as it were in various directions from a focus of reflex-discharge which takes effect on the limb itself."

Before describing the results of our experiments, it will be well to summarize the method which is described in full in our preceding paper, and to describe such additional procedures as were employed for the purpose at hand.

The flexion-reflex in the decerebrate cat has been examined with the string galvanometer by the following method. Decerebration under profound ether anaesthesia has been performed in the usual way.[5] The essential features of the operation are the ligation of both carotid arteries and the removal of the entire cerebrum above the level of the posterior corpora quadregemina. A preparation is thus obtained which remains in decerebrate rigidity without manifesting other muscular action unless stimulated. For stimulation a pair of Sherrington shielded electrodes was applied to the popliteal nerve cut distal to the point of stimulation. These were connected with the secondary coil of a Berne inductorium in such a way that break shocks should be ascending (i.e., with cathode central). The coil (designated coil *F*) which is 14 cm. long, was calibrated in accordance with Martin's method,[6] and the current in the primary coil was measured with an ammeter. The flexion-reflex was thus elicited by means of single induction shocks of known value applied directly to an afferent nerve. The action current of the motor nerve (peroneal) supplying the tibialis anticus muscle in the same leg was led off with a pair of non-polarizable "boot" electrodes and recorded photographically from the string galvanometer. In most cases the nerve was crushed between the leading-off electrodes to render the action currents monophasic. In almost all cases the peroneal nerve was subsequently severed at the hip and stimulated directly just distal to the point of severance while the leading-off electrodes remained in position, in order to obtain for comparison records of the action currents under direct stimulation.

Most of these experiments also supplied the results to be reported in the present paper. The principal procedures employed for the purposes of the latter and not already described were the variation over a wide range of the strength of stimulus used to evoke the reflex and the subsequent recording of monophasic responses directly from the

[5] See Forbes and Sherrington: This Journal, 1914, xxxv, 367.

[6] Martin: Measurement of Induction Shocks, New York, 1912, 55.

afferent nerve with a similar series of stimuli. Further details of procedure will be described as the need arises.

In describing the results it will be well to begin with the action currents led off from the afferent nerve in response to induction shocks of graded intensity. Few experiments seem to have been reported dealing with the gradation of electrical responses in nerve trunks. Waller in 1895[7] applied tetanizing stimuli to a frog's sciatic nerve for periods of one-eighth minute each and recorded simultaneously the mechanical lift of the innervated muscle and the "negative variation" of the demarcation current at the central end of the nerve. The negative variation was recorded with a slow moving galvanometer which showed by a single excursion the whole series of electrical responses resulting from the continued tetanization. He reports that on grading the intensity of stimuli in a series of such observations the negative variation increases in proportion to the stimulus even after maximal contraction is obtained. His statement is: "On nerve the curve expressing the relation between cause and effect is a straight line, the effect is proportional to the cause, at least within moderate ranges of excitation, exceeding, however, the upper limit of maximum functional effect, as gauged by muscular contraction." He does not appear, however, to have extended his observations to stimuli much more than double the threshold value.

Lucas[8] more recently showed evidence which favored the view that in a single skeletal muscle fiber, excited through its motor nerve, the contraction follows the "all-or-none" law. But in commenting on his results he pointed out that this conclusion "does not imply that the nervous impulse is always maximal any more than it implies that the electric current used to excite the nerve is always maximal." He further remarks that the observation of Waller mentioned above does not, as might at first appear, prove gradation of response in the individual nerve fiber, since muscular contraction was in this case recorded in the gastrocnemius, and there are many fibers in the sciatic nerve which do not innervate this muscle. These might have been responsible for the increment in the negative variation after the muscular contractions had become maximal. It should also be noted that in Waller's experiment the leading-off electrodes were at the central end of a frog's sciatic nerve and the stimulating electrodes were placed midway be-

[7] Waller: Brain, 1895, xviii, 208.

[8] Lucas: Journ. Physiol., 1909, xxxviii, 132.

tween these and the muscle. Thus the stimulating electrodes cannot have been more than 3 or 4 cm. at most from the leading-off electrodes. We find evidence, to be described presently, indicating that with strong induction shocks electrical disturbances may appear in the recording instrument which seem to be distinct from the action current; and at such close proximity to the stimulating electrodes their effects may be considerable. A slow moving galvanometer could not have differentiated between different kinds of electrical effect. It seems, then, that Waller's experiment does not prove the possibility of gradation of electrical response in a single nerve fiber, nor does it show that the electrical response of a whole nerve trunk increases without limit in response to increase in the strength of the stimulus. Lucas concluded his discussion of the subject with the following statement: "We must therefore regard the question whether the response of a nerve fiber is capable of gradation as being at present undecided."

Gotch[9] in 1902 studied the electrical response in nerve to a single induction shock with a capillary electrometer. His chief concern was the comparison of the time relations in maximal and submaximal responses. He gave no detailed statement concerning the gradation of magnitude in the responses. He estimated the strength of shock requisite for minimal and maximal stimulation by means of the resulting muscular contraction. In order to obtain both submaximal and maximal responses he made his experiments "with intensities of induction shocks starting slightly weaker than the above minimal value and increasing to beyond that of the maximal one;" how far beyond the maximal value is not stated, but apparently the entire range of intensities was not great. The only statement in Gotch's paper in regard to the gradation of magnitude in the electrical response is the following:[10] ". . . . . it seems clear that the electrical response of nerve to a single stimulus varies as to its magnitude in correspondence with variations in the exciting efficiency of the stimulus, and that, like muscle, the response of nerve passes through a series of submaximal stages of increasing value until a maximum is reached." So far as we are aware the fact here stated, that a limiting maximal value of the electrical response in nerve is reached as the stimuli are increased in strength, has not been confirmed by other observers, nor did Gotch place more than passing stress on the fact or record accurate measurements with stimuli of widely varying intensity.

[9] Gotch: Journ. Physiol., 1902, xxviii, 395.

[10] Loc. cit: 405.

Gotch found the time relations in maximal and submaximal responses to be identical, and inferred that such gradation of magnitude as occurred signified chiefly if not wholly a gradation in the number of fibers excited, and that the individual nerve impulse probably obeys the "all-or-none" law. Quite recently Adrian[11] has shown that when a nerve is gradually narcotized, muscular contraction being taken as the index of response, a stage is reached just before conduction ceases altogether at which an "all-or-none" relation of response to stimulus is directly demonstrable. By certain ingenious control experiments he seems to have excluded the possibility of arriving at any other conclusion than that the nerve impulse is unaffected by variation in stimulus, i.e., that it obeys the "all-or-none" law. If this be so, and if the action current be a true index of physiological activity, incapable of varying independently of the nerve impulse it denotes, then it is obvious that a limiting maximal value of the action current must be reached when all the fibers in a nerve trunk are excited.

We can see no other explanation of Adrian's observations than the assumption of the "all-or-none" relation, but in view of the far-reaching consequences of this assumption it seems wise to consider the possibility that some other unthought-of explanation may yet be found when the problem is viewed from a wholly new angle. There is also the possibility which Gotch has suggested[12] that the electrical response is not an infallible criterion of physiological activity; in particular, it is conceivable that quantitative differences in the action current might exist where none existed in the activity as judged by any true functional criterion. For instance, even though the propagated disturbance, as judged by its ability to induce contraction in the innervated muscle or by the distance it can travel through a narcotized portion of the nerve,[13] cannot be varied by any variation in the stimulus, the electrical response might be capable of gradation.

II. EXPERIMENTAL RESULTS

A. Correlation between stimulus and action current in the nerve trunk.

Since we find on record no direct proof that the action current in a nerve trunk cannot increase in magnitude beyond a limiting maximal

[11] Adrian: Journ. Physiol., 1914, xlvii, 460.

[12] Gotch and Burch: Journ. Physiol., 1899, xxiv, 422; Gotch: Journ. Physiol, 1902, xxviii, 51.

[13] Cf. Adrian: Journ. Physiol., 1912, xlv, 393.

value however strong the stimulus, we have deemed it worth while to examine this question with some care. Most of our experiments on this point have been made with the cat's popliteal nerve severed at hip and knee, stimulated at the peripheral end and led off at the central end. The reason for this was that we regularly used this nerve to induce reflex responses, and it was desired to examine with a view to gradation the afferent impulses involved in their production, and to do so as soon as possible after the reflex responses had been recorded and under identical conditions of stimulation. Rather than introduce the delay and probable shift of electrode contacts incidental to transferring the nerve to a moist chamber after removing it from the animal's body, the central end was usually laid across a pair of non-polarizable "boot" electrodes in the open air, the stimulating electrodes were kept in as nearly as possible the same position on the nerve and the observations were made as rapidly as possible in order to minimize the effects of drying and other progressive changes which would invalidate the measurements. Some experiments were made on the peroneal nerve, that portion on the leading-off electrodes being in the moist chamber used for recording reflex responses and already described in our preceding paper. In some experiments the whole sciatic nerve was used and in one it was placed in a moist chamber. In all cases the nerve was dissected out by cutting the surrounding fascia with a sharp knife or scissors, and in all cases it was crushed approximately midway between the leading-off electrodes to render the responses monophasic. The platinum stimulating electrodes were so applied that on the break shock the kathode should be nearest the leading-off electrodes; and for quantitative observations break shocks were always used. In this way the possibility of electrotonic block was avoided. The distances between electrodes were kept constant throughout the course of each experiment, except in specified cases when it was purposely varied.

Accurate comparison of the magnitudes of successive electrical disturbances is rendered difficult by the following progressive changes which tend to alter experimental conditions:*

1. The string galvanometer is gradually heated by the passage of the current used to create the magnetic field. The string has not the same coefficient of expansion as the case in which it is held, and its tension therefore undergoes a progressive change. This, in turn, changes the magnitude of excursion resulting from a given electrical disturbance.

* In addition to the changes enumerated below, the gradual cooling of a nerve on removal from the animal's body should be mentioned.

This source of error can be minimized by frequently readjusting the tension, but the resulting delay serves to increase the error due to the other progressive changes.

2. Progressive drying of the nerve acts in two ways: (a) it increases the total resistance in the secondary circuit, thereby reducing the current produced by a given difference of potential; (b) it eliminates the short circuit provided for the action current by moisture on the surface of the nerve; for the greater the layer of conducting fluid on the nerve, the smaller must be the proportion of the total action current which goes through the string. Of these two factors acting as the nerve dries (a) tends to reduce the excursions of the string, (b) simultaneously tends to increase them.

3. Any injury to the nerve through trauma or drying may introduce a region of impairment where the impulses undergo decrement,[14] and may be abolished altogether in some of the fibers. Such a region of impairment seems to extend for some distance along a nerve from the point where a ligature is applied. Even in a moist chamber we have found the magnitude of the action currents to decrease considerably in less than half an hour if the stimulus was applied near the ligatured end, although the strength of stimulus and all other experimental conditions remained unchanged. We have further found that stimuli applied near where a nerve has been ligatured do not produce nearly so large action currents as when applied far from the seat of injury. Experiment 28 was a striking example of this. The sciatic nerve being ligated and cut at the knee, was stimulated centrally and the reflex responses were noted. An hour after the nerve was ligated it was severed at the hip and crushed as usual in order to record afferent impulses monophasically. It was then placed in a moist chamber; stimulating electrodes were applied about 15 mm. central to the ligature and leading-off electrodes central to these. The nearest lead was about 50 mm. from the point of stimulation. With this arrangement the largest excursions of the string in response to the action current amounted to 4.0 mm.[15] The stimulating electrodes were then shifted about 23

[14] See Adrian: both references cited above; also Lucas: Journ. Physiol., 1913, xlvi, 470. Gotch (Journ. Physiol., xxviii, 55) mentions hyper-excitability as occurring in nerve near the seat of a recent injury; this hyper-excitability had probably passed off in our experiments before the observations were begun.

[15] The figures given for excursions of the string refer, of course, to the magnified shadow of the string as appearing on the photographic film. The magnification in all cases was 580 diameters, as stated in our previous paper.

mm. nearer the leads without disturbing the contacts with the latter, and the action currents from maximal stimuli then amounted to about 5.1 mm.

On account of these various sources of error it was difficult to determine with accuracy the gradation of electrical disturbance in response to graded stimuli or to ascertain whether a definite limiting maximal value is reached. Still, by making observations in rapid alternation between two stimuli of different strength, it was possible to nullify almost completely the progressive sources of error, and to obtain valid comparisons. With regard to the effect of stimulating in a damaged portion of the nerve (e.g., close to a ligature), besides such error as is due to the progressive character of the impairment, we should expect additional disturbance of comparisons even with a minimum lapse of time. For, in the first place, the thresholds of those fibers suffering most from injury would probably be much above normal and would differ far more from those of the least injured fibers than in normal nerve; in the second place, it seems probable from the figures just given (Experiment 28) that in such a case some fibers are wholly inexcitable at the point of stimulation, but if strong enough shocks are employed the electrical disturbance will spread along the nerve to a point where the fibers are still excitable. From both these causes it should result that the limiting maximal value, if there be such, is only reached with stronger stimuli than in the case of uninjured nerve, and therefore the gradation of response should be extended over a wider range of stimuli than normal. As will presently appear, our records seem to show just such an increase in gradation when impairment has been present. Consequently, the failure in a given case to find a limiting maximal value below the point where the shocks are so strong that they manifestly introduce non-physiological factors, does not prove that a limiting value is not normally present. On the other hand, any case in which, under favorable conditions, a limiting maximal value is consistently found can be regarded as valid evidence that such a limit to the intensity of electrical disturbance in nerve exists. Any other inference must involve the assumption of some highly improbable coincidence.

We have examined many nerves with the method described, but, for the reasons just given, only a few experiments have afforded grounds for conclusions. In grading electrical responses it must be remembered that the action current in nerve is far too brief to be followed accu-

rately by the string.[16] It is not safe to assume that the excursion of the string is even directly proportional to the maximum difference of potential between the leads; but it is safe to assume that as long as all other conditions remain strictly parallel equal excursions denote equal electromotive forces, and an increase in the excursion denotes an in-

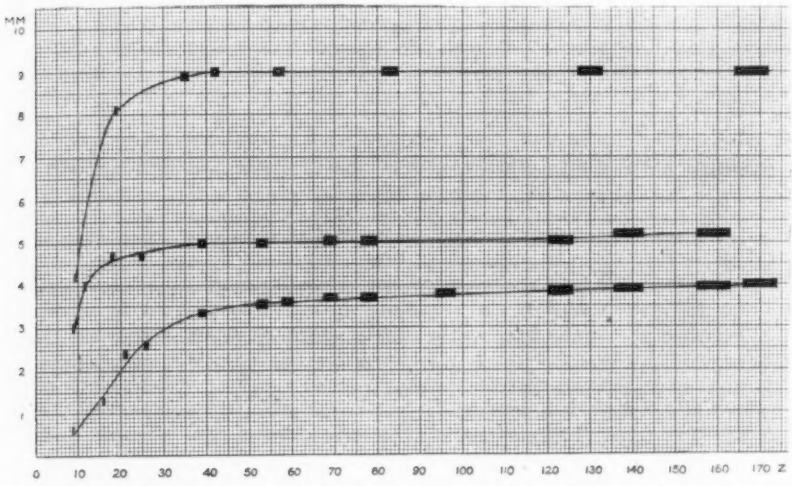

Fig. 1. Relation of monophasic action current to stimulus. Excursion of string in millimeters (ordinates) plotted against Z units (abscissae); only break shocks used. The rectangles mark the limits of experimental and observational error (assuming 2.5 per cent as the possible deviation from the given value of Z).

Uppermost curve: Experiment 21, sciatic nerve.

Lowest curve: first half Experiment 28, sciatic nerve.

Middle curve: second half Experiment 28, same nerve (see text).

The numbers assigned to experiments in this paper correspond to the numbers of the preparations given in our previous paper, the same preparations usually furnishing data for both papers. They are numbered mostly in chronological order, but some early experiments relating only to this paper are numbered out of sequence.

crease in electromotive force. Our chief concern has not been to establish any exact mathematical relationship between stimulus and response, but to determine within what limits gradation of response occurs, i.e., whether a limiting maximal value is reached, and, if so, at about what strength of stimulus. Therefore, the magnitudes of ex-

[16] See Forbes and Gregg: Loc. cit., figure 7, 146.

cursion furnish all the data needed for the desired comparison of electrical disturbances. Thresholds were not accurately recorded since we were concerned chiefly with the approach to maximal stimulation.

The relation between response and stimulus over a wide range is shown in figure 1 (middle curve) taken from Experiment 28, one of the few in which conditions can be considered normal. The measurements are all taken from the second half of the experiment after the stimulating electrodes had been moved to a point 38 mm. from the ligature; the nerve was in a moist chamber and the observations were all made in the course of about twenty minutes. Stimuli of certain strengths were repeated several times, sometimes both before and after weaker or stronger stimuli; in this way approximate constancy of con-

TABLE 1

Z UNITS	MM. EXCURSION	Z UNITS	MM. EXCURSION
83	9.1	83	8.9
139	9.1	467	(9.0)
130	9.0	83	9.0
230	(9.2)*	35	8.9
83	9.0	35	8.9
57	9.0	19	8.1
57	9.0	9.5	4.2
168	(9.0)	42	8.9
168	(9.0)	83	8.9
330	(9.2)		

* Parenthesis means that at this coil distance the make shock record showed deformation.

ditions was shown. The strength of stimulus is given in Z units on the Martin scale. The magnitude of excursion is given in millimeters and the measurements are accurate to within about 0.1 mm.

It will be seen that from 39 Z to 123 Z the excursion remains constant between 5.0 mm. and 5.1 mm., the recorded variation lying within the limits of observational error. At 139 Z and 159 Z excursions of 5.2 mm. are recorded. However, at values not much above this we find evidence, to be discussed presently, that electrical disturbance other than the usual simple action current is being recorded. It seems reasonable, therefore, to conclude that the slight increase in the magnitude of excursion occurring with stimuli stronger than 123 Z does not

signify an increase in the magnitude of the action current, and that this experiment shows a limiting maximal value.

Experiment 21, in which the whole sciatic nerve was used for reflex stimulation and then for recording afferent impulses, showed a limiting maximal value even more clearly than Experiment 28, just described. In this the series of action current records was begun less than half an hour after the nerve had been exposed and ligated, and the stimulating electrodes were applied about 27 mm. from the nearest lead; the distance from the ligature was not accurately recorded, but it was considerably more than 2 cm. Table I shows measurements in chronological order from nineteen observations taken in rapid succession; beside each is shown the strength of stimulus. It is evident that a slight progressive diminution of the excursions occurred during this series. Correcting for this as nearly as possible the relation of response to stimulus will be found to follow the uppermost curve in figure 1. Constancy of response occurred here over a wider range of stimulation strengths than in Experiment 28, and, furthermore, this constancy persisted even beyond the point at which the records showed deformation. Another preparation showed no increase in the response of the peroneal nerve between 71 Z and 197 Z; still another showed practically no increase between 9.5 Z and 210 Z in the case of the peroneal nerve. Another peroneal nerve, which yielded the largest action currents obtained in any of our experiments, showed an increment of not more than 1 per cent between 13.6

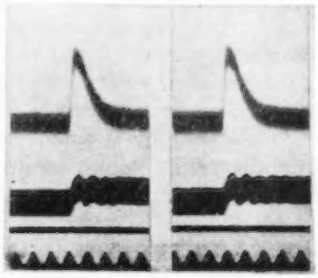

Fig. 2. Monophasic action currents, peroneal nerve. Experiment 32.

Stimuli, break shocks (coil *F*), in A, 13.6 Z; in B, 23.4 Z.

String D (see previous paper, p. 122).

In this and all other photographic records the top line shows the excursions of the string. The second line shows the time of stimulation (except when the signal magnet was disconnected). A fall in this line shows the make, a rise the break of the primary current. The small oscillations following the break are vibratory and do not indicate secondary closure of the circuit. The bottom line records time; each complete vibration = 0.01 second. In all nerve records upward excursion of string means fall of potential in proximal lead. In all experiments the nerve impulse was blocked between leads to render the action current monophasic.

Z and 23.4 Z, the records, two of which are reproduced in figure 2, being taken within about a minute of each other.[17]

Certain other experiments yielded curves in which the general shape closely resembles those already discussed, but in which after the approximate maximum was reached a very slight increase in the excursions persisted with further increments in the strength of stimulus. That these do not invalidate our conclusion as to a limiting maximal value is shown by an examination of Experiment 28. In this instance stimuli applied within 15 mm. of the ligature produced just such a continued gradation of excursions after an approximate maximum was reached. This is illustrated in the lowest curve in figure 1. Here an increment of response was found between 97 and 170 Z. At 558 Z no more increment appeared. Gradation here persisted up to the point at which measurements cannot be trusted because of the deformation. It has already been noted that in this experiment when the stimulating electrodes were moved 23 mm. farther from the ligature excursion of 5.0 to 5.1 mm. were obtained with all stimuli of more than 39 Z. It is quite evident that even with 558 Z applied in the region of impairment maximal stimulation of the nerve trunk was not obtained. It is presumable that a considerable number of fibers could not be excited at all in this region. The persistent gradation found in stimulating here was probably due to the great rise of threshold in many of the fibers, and perhaps also to the fact that some of the fibers couldn't be stimulated at all in this region, but only when the shocks were so strong that the spread of current reached a sufficient value at the nearest point where excitability remained.

We feel, therefore, that the conclusion is warranted that in fresh uninjured nerve a limiting maximal value to the action current is reached when the stimulus attains a certain strength. The strength of stimulus barely sufficing to give the maximum response in normal nerve was found, in the two preparations affording the best grounds for conclusions, to be in the neighborhood of 40 Z units. In the majority of our experiments impairment seems to have occurred and a very slight gradation persisted above the approximate maximum. This approximate maximum in such cases usually occurs in the neighborhood of 50 or 60 Z units. Although the value given above (40 Z) is probably the

[17] In addition to these experiments done in this laboratory one of us performed a similar experiment on a cat's peroneal nerve with Dr. H. B. Williams, in the Physiological Laboratory of the College of Physicians and Surgeons in New York. This experiment also gave a result similar to those recorded here.

normal maximal value for the uninjured nerve trunk, it must be remembered that in most physiological researches nerves are subjected to a good deal of pressure in dissection, especially when blunt instruments are used, and are often ligated near the point of stimulation. In such work, therefore, electrical recording would probably show a condition more like the majority of our experiments, with a higher approximate maximal value of stimulus and a somewhat persistent grading of response.

B. Deformation of the action current record

In our experiments we have found the galvanometer records to show only the characteristic curve of the action current with both make and break induction shocks as long as these shocks were kept below a certain range of values (150–300 Z units in the case of break shocks); but with stronger shocks the curves have shown deformation which becomes increasingly marked as long as the strength of the shocks is increased. Figures 3 and 4 show several series of such irregularities, together with normal submaximal and maximal responses for comparison. Make as well as break shocks are shown, and it will be noted that the former cause deformation usually at more remote coil-distances than the latter, although the make shocks under the conditions obtaining in the primary circuit are about one-eighth as powerful physiologically as those of break shocks at the same coil-distances, if we may rely on evaluation by thresholds.[18] In view of the approximate uniformity of experimental conditions,[19] the variety presented by the records is rather striking. In some it will be seen that the deformation consists chiefly in a sharp preliminary notch (e.g., figure 3, row B, No. 4, row *C*, No. 5 break shocks) similar to that shown in some of Gotch's capillary electrometer records. In some few of the records (e.g., figure 3, row B, No. 3; figure 4, lower row, No. 3) the only anomaly consists in a second excursion of the string, suggesting as its cause a second impulse in the nerve. Garten has made a similar observation which he interprets in this way.[20] Theoretical reasons for assigning some degree of probability to this interpretation will be considered further on. Several of the records are too complex to be analyzed without far more data than we have been able to secure. We have obtained some evidence, however, which tends towards their elucidation.

[18] See Martin: Op. cit., 102.

[19] Exception to this is noted below and in the legends to figures 3 and 4.

[20] Garten: Zeitschr. f. Biol., 1909, lii, 552, and figure 17.

a. The electrical artefact. In the literature of electro-physiology it is not uncommon to find mention of the "escape of current" into the recording apparatus. For instance, Gotch and Burch,[21] referring to their records of the action currents of nerves taken with the capillary electrometer, mention sharp notches in the curves as "due to an escape

Fig. 3. Simple and deformed action current records compared. Each horizontal row shows a series of observations from a single experiment without shift of electrode contact; each space between records denotes change of coil distance; stimuli are progressively stronger from left to right in each row. Two speeds of film were used. To aid comparison some records at both speeds, otherwise identical, are put side by side. In all except row D (as in all other nerve records in this paper) the proximal lead was connected with the upper end of the string. In this case the leads were reversed; also the magnet current.

In the following, each group of records at one coil distance is designated with a single number; coil distances are given in millimeters, Z units refer to break shocks (make shocks not evaluated). Coil *F* used in all. Current in primary coil given in amperes (the same throughout each experiment). The response was rendered monophasic by crush in all but Experiment 5.

Row A, Experiment 5, peroneal nerve. String C. High-voltage magnet coil. Nerve devitalized by heat under distal lead.

Shunt in primary circuit (see previous paper, p. 138), 0.105 amp. Martin knife-blade key. 1, 250 mm., $\frac{11}{K}$ Z; 2, 150 mm., $\frac{99}{K}$ Z; 3, 90 mm., $\frac{465}{K}$ Z; 4, 80 mm., $\frac{506}{K}$ Z; 5, 70 mm., $\frac{538}{K}$ Z; 6, 40 mm., $\frac{598}{K}$ Z; 7, 0 mm., $\frac{662}{K}$ Z.

Row B, Experiment 8, peroneal nerve. String D. High-voltage magnet coil. No shunt in primary circuit; 0.226 amp. Knife-blade key. 1, 370 mm., 6.8 Z; 2, 160 mm., 164 Z; 3, 120 mm., 650 Z; 4, 0 mm., 1580 Z.

Row C, Experiment 9, peroneal nerve. String D. High-voltage coil. 0.284 amp. Knife-blade key. 1, 360 mm., 9.5 Z; 2, 160 mm., 210 Z; 3, 140 mm., 425 Z; 4, 100 mm., 1260 Z; 5, 0 mm., 2020 Z.

Row D, Experiment 17, popliteal nerve in moist chamber. String D. High-voltage coil. 0.222 amp. Copper point and mercury key (see previous paper, p. 132). 1, 360 mm., 7.4 Z; 2, 180 mm., 93 Z; 3, 150 mm., 236 Z; 4, 140 mm., 334 Z; 5, 0 mm., 1570 Z.

Row E, Experiment 21, sciatic nerve. String E. Low-voltage magnet coil. 0.306 amp. Copper point and mercury key. 1, 370 mm., 9.5 Z; 2, 180 mm., 130 Z; 3, 170 mm., 168 Z; 4, 140 mm., 467 Z; 5, 80 mm., 1690 Z; 6, 50 mm., 1950 Z; 7, 0 mm., 2200 Z.

The first four break shocks in Row E furnished measurements recorded in Table I.

For calibration curves, see figure 5. The knife-blade key when used in these experiments gave clean makes and breaks.

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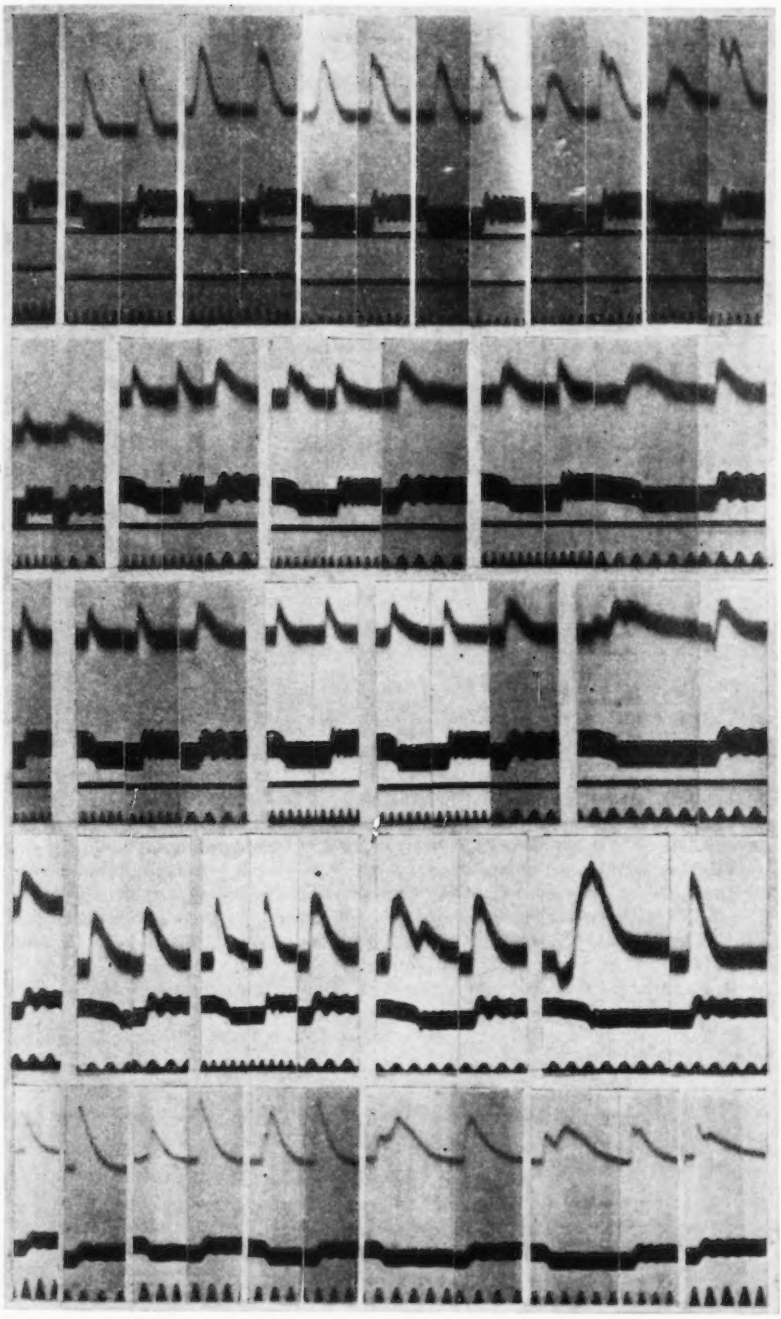
[21] Gotch and Burch: Loc. cit., 413.


A
B
C
D
E

from . . . . the induction shocks used for excitation." Lucas has used such notches as evidence of the exact time of stimulation.[22] The exact nature of this "escape of current" is not stated in those papers in which we have found it mentioned, but it is our impression that many physiologists understand the term to imply a condition in

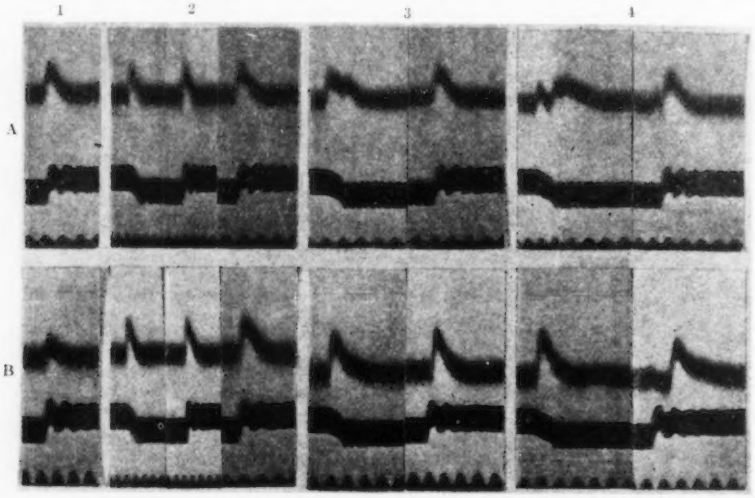


Fig. 4. Same plan of arrangement as in figure 3. Coil *F*. String D. High-voltage magnet coil. In A the kathode of the secondary coil on break shock was connected with the stimulating electrode nearest the galvanometer leads, as was the case in all experiments when not otherwise stated. In B the stimulating electrodes were reversed. The difference between the two experiments supplements the evidence in figure 15.

A, Experiment 30, popliteal nerve. 0.280 amp. Knife-blade key. 1, 310 mm., 15.6 Z; 2, 180 mm., 119 Z; 3, 140 mm., 425 Z; 4, 0 mm., 2000 Z.

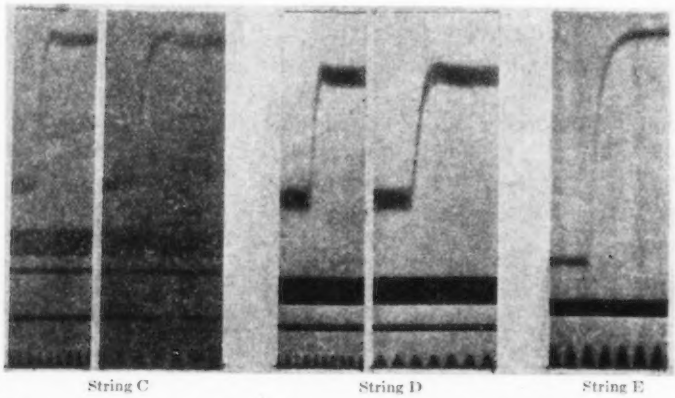
B, Experiment 31, sciatic nerve removed under ether, kept 3 hours in Ringer solution, room temperature; then in moist chamber 4 hours before observations. 0.278 amp. Knife-blade key. 1, 250 mm., 32 Z; 2, 160 mm., 207 Z; 3, 120 mm., 816 Z; 4, 0 mm., 1990 Z.

which the leading-off electrodes are so placed in relation to the tissue that the recording circuit can provide a path for a portion of the current which passes between the two stimulating electrodes as in figure 6. That this does not represent the distribution of electrical disturb-

[22] Lucas: Journ. Physiol., 1911, xliii, 51 and figure 2, 52.

ance in such arrangements as we have dealt with, we have both theoretical and experimental evidence.

It is not necessary that the recording circuit shall provide a path for the current flowing between the stimulating electrodes, nor that one of the leads should be very close to one of these in order that appreciable electrical disturbances resulting directly from the stimulating cur-



String C String D String E

Fig. 5. Calibration curves. These show typical excursions of the three strings employed, on the make of a constant current. The tension in the case of Strings C and D was the same as was regularly employed in all experiments with the string in question (see previous paper, p. 122); in the case of String E it was the same as in the records in figure 3, E. The resistances were of the same order of magnitude as was commonly found in our experiments; with String D it was identical with that found in the nerve circuit used for figure 3, B. The E.M.F. and resistance in series with the string in each were as follows.

String C: $\frac{10 \text{ millivolts}}{\text{string}+46{,}000 \text{ ohms}}$ String D: $\frac{10 \text{ millivolts}}{\text{string}+38{,}000 \text{ ohms}}$

String E: $\frac{10 \text{ millivolts}}{\text{string}+25{,}000 \text{ ohms}}$

rent may appear in the recording instrument. It is an elementary fact in electricity that if the potential of any point on a conductor is suddenly altered a transient current flows between that point and the rest of the conductor, tending to equalize the potential throughout. This current at any point varies in magnitude according to the electrostatic capacity to which the conducting path under consideration leads. Every conductor has some capacity and any portion having large ca-

pacity will provide a sort of reservoir to which, under the conditions assumed, an appreciable current will flow. Now it has been pointed out by Williams and Crehore[23] that a nerve trunk, on account of its structure, must have considerable electrostatic capacity. Furthermore, most pieces of recording apparatus have appreciable capacity; this is notably so in the string galvanometer of the Cambridge make (used by us), in which one end of the string is connected with the core of the magnet. When, therefore, the potential of a nerve is suddenly lowered[24] at the point of stimulation a transient current flows to this point from all remote portions of the nerve and from such capacity as may be connected thereto in the form of recording apparatus. The general distribution of lines of current flow may be indicated schematically in figure 7. In determining the nature of the transient current through the string the distribution of capacity in various parts of the connected system of conductors is therefore of considerable importance.

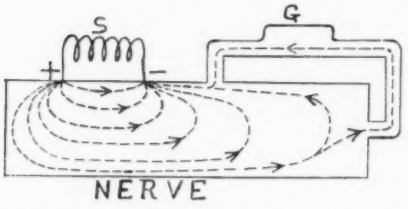


Fig. 6. *S*, stimulating inductorium; *G*, recording instrument (galvanometer or electrometer).

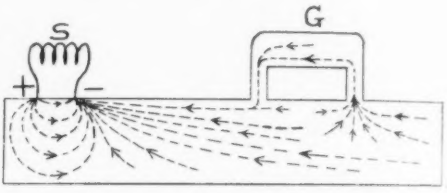


Fig. 7. *S*, stimulating inductorium; *G*, galvanometer. Capacity is roughly represented by enclosed area.

Referring to figure 7 in which is shown a rough schematic representation of the capacities involved in our experiments, it will be noted that there is capacity distributed throughout the length of the nerve trunk as well as in the galvanometer. The capacity of the galvanometer is chiefly in the iron core and this is metallically connected with the upper end of the string. In all of our experiments with nerves under direct stimulation, except that recorded in the fourth row (D) of figure 3, the proximal lead (i.e., the electrode on the nerve nearest to the stimulating electrodes) was connected with the upper end of the string, the distal lead with the lower; in this one case the usual connections were reversed as was also the current exciting the galva-

[23] Crehore and Williams: Proc. Soc. for Exper. Biol. and Med., 1913, xi, 59.

[24] We choose as our example the lowering instead of the raising of potential, since this is what occurs at the stimulating electrode.

nometer magnet.[25] Thus in all but one of the experiments shown in figures 3 and 4, the galvanometer capacity was proximal to the string. much depends on the position, in relation to the electrodes, of the maximum capacity of the nerve trunk. Thus, if the capacity of that portion lying beyond the distal lead be far greater than that of the galvanometer, the transient current will flow through the string in the direction indicated in figure 7 (assuming the potential of the stimulating electrode nearest the lead to have been lowered). If the greater part of the total capacity be in the magnet core, the transient current will flow in the opposite direction through the string. Briefly, the direction of the transient currents in the string depends on whether the capacity of that portion of the nerve which is more accessible to

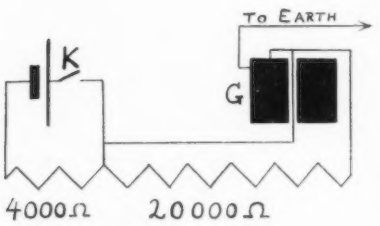


Fig. 8. *K*, key; *G*, galvanometer.

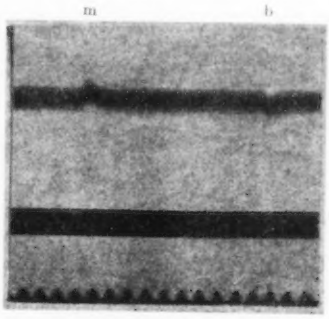


Fig. 9. Transient effects from make (*m*) and break (*b*) of constant current. String D. Wiring shown in figure 8. Platinum contact key.

the spreading current by way of the galvanometer circuit than by way of the nerve itself bears more or less than some fixed ratio to the capacity of the magnet core. Thus, we might expect to find the transient effect occurring in opposite directions in different experiments unless the nerves are always identical in size and capacity and identically arranged.

In order to show the presence and nature of the transient currents in circuits uncomplicated by action currents, two methods were employed;

[25] This was reversed in order to make the action current deflect the string in the usual direction.

one was the substitution of blank resistances for the nerve, making these of the same order of magnitude as is commonly found in nerve, and the other was the use of a dead or inactive nerve in place of an active one. The experiments with the blank resistance will be described first.

The simplest condition studied was that of a galvanic current derived from a single galvanic cell. A 4000 ohm spool was connected with a dry cell and a key; a 20,000 ohm non-inductive resistance was connected with the galvanometer terminals; the string terminal not connected with the magnet core was also connected with one end of the 4000 ohm spool; the magnet core was put to earth. The arrangement is shown in figure 8. Making and breaking the circuit through the 4000 ohm spool caused transient excursions of the string of which examples are recorded in figure 9.

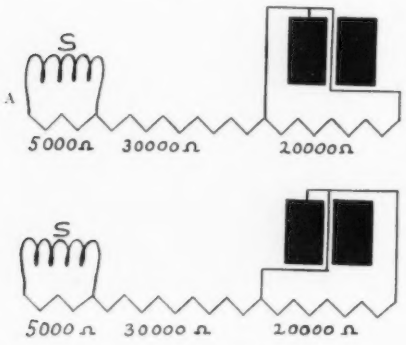


Fig. 10. *S*, secondary coil of inductorium.

Conditions more closely resembling those obtaining in our experiments were studied by replacing the dry cell with an inductorium. In this experiment a small 5000 ohm spool was introduced between the terminals of the secondary coil. The 20,000 ohm segment of a non-inductive resistance box was connected with the terminals of the galvanometer, and the adjacent 30,000 ohm segment of the same box was used to connect one or other terminal of the galvanometer with that terminal of the secondary coil known to be kathode on the break shock. Figure 10 shows the arrangement of apparatus in this experiment. *A*, with the upper end of the string proximal, represents the usual condition in our experiments with nerves. *B*, with the wires reversed, represents the arrangement in Experiment 17 (shown in fig. 3, D) with the exception that in the experiment with the blank resistance the current exciting the magnet was not reversed as it was in Experiment 17. Figure 11 shows the excursions of the string resulting from make and break shocks, with the secondary coil at zero (i.e., completely covering the primary), in both arrangements of the circuit, and in each case

with and without the magnet core being put to earth.[26] The excursions differ from those obtained with a galvanic cell in that they show, as is to be expected, that the transient current is diphasic, i.e., flows first in one direction, then in the other through the string. When the core is not to earth the excursions differ in the two arrangements chiefly in that they are in opposite directions, but they also differ slightly in size, that with the upper end of the string distal being the larger. When the magnet core is put to earth this difference in magnitude is greatly increased, the excursion being almost abolished when the earthed end of the string is proximal and much augmented when it is distal. The initial deflection in each case indicates that the potential of the end of the string connected with the proximal lead has changed in the same

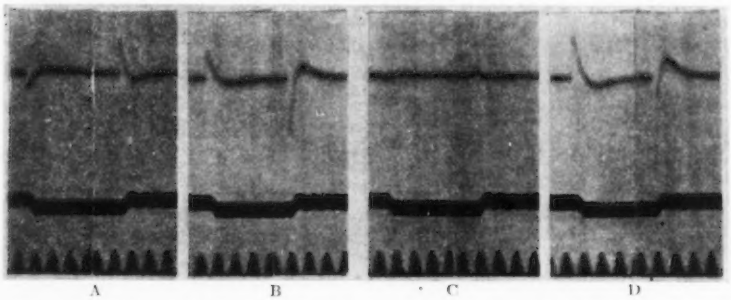


Fig. 11. Diphasic transient effects from induction shocks. In A and C the wiring was as shown in figure 10, A; in B and D it was as in figure 10, B. In A and B the magnet core was not put to earth; in C and D it was. String E. Tension, 1 cm. excursion = 12.4×10^{-8} ampere; i.e., twice that used in the calibration curve (fig. 5). Copper point and mercury key.

sense as has the terminal of the secondary coil nearest to the galvanometer; i.e., the end of the string nearest the induction coil is negative when the negative terminal of the inductorium is nearest the string. The difference in magnitude between the excursions in *A* and *B* (fig. 11) depends upon the capacity of the magnet core. An important difference between the electrical arrangements in this artificial system

[26] In this and all subsequent experiments the galvanometer was equipped with the low-voltage magnet coil excited by 8 Edison storage cells. In the experiment shown in figures 8 and 9 the high-voltage magnet coil was used and was excited by the current from the local power plant. Cf. Forbes and Gregg: Loc. cit., 121.

and in those involving a nerve trunk is that with the nerve non-polarizable "boot" electrodes are interposed between the nerve and the galvanometer, and these have resistances of several hundred ohms, often over a thousand. The significance of this is that the if the principal capacity were in the magnet core no appreciable current would flow to it by way of the string in a system such as is shown in figure 10, *A*, for on the principle of divided circuits practically all the current would flow through the proximal lead wire which has a negligible resistance compared with the other path. On the other hand, with an electrode resistance such as is interposed in a physiological circuit, an appreciable fraction of the total transient current would flow through the string on its way to the core.[27]

The other method used for examining these electrical disturbances, which we may for convenience call "artefacts," was the introduction of nerves which were dead or at least physiologically inactive. Three such were studied. One was a peroneal nerve which after removal from the animal's body was kept for two days in Ringer solution at room temperature, after which it showed no trace of action current on stimulation. Another nerve was removed from an animal immediately after decerebration under excessively profound ether anaesthesia, and for some reason failed to exhibit any action currents during the next hour when it was examined. That the nerve was not dead was shown by the fact that after it had been washed for over an hour in Ringer solution it exhibited action currents which were nearly normal. The third nerve (popliteal) was killed by immersing it in Ringer solution heated to 70°C.

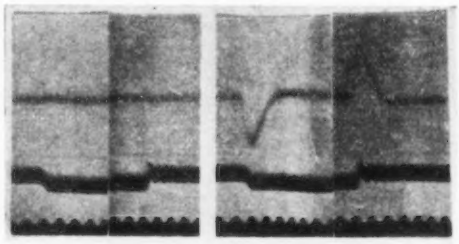
The first of these nerves had yielded normal action currents when fresh, and after 19 hours in Ringer solution, yielded action currents still more than half as large as when fresh. After 44 hours in Ringer solution it was arranged with 4 cm. intervening between the stimulating electrodes and the nearest lead to the galvanometer. No excursion of the string occurred until the secondary coil was brought close enough to give a break shock of 620 Z units. This produced an excursion barely discernible in the record. Make and break shocks with the secondary at zero (break shock, 2000 Z units) produced excur-

[27] One experiment was performed with graphite lines on ground glass for resistance, and a galvanic cell instead of an induction coil, and the resistances were arranged to be closely comparable with those of nerve and electrodes as ordinarily set up. No excursions at all resulted from opening or closing the galvanic circuit; probably because the capacity of the conductor was too small.

sions shown in figure 12, *A*. The stimulating electrodes were then moved to within 12 mm. of the proximal lead and shocks of the same strength produced the greatly augmented excursions shown in figure 12, *B*.

The nerve whose functional activity was temporarily suspended was arranged with the galvanometer leads 35 mm. apart (the usual distance was about 25 mm.), and the stimulating electrodes only 15 mm. from the proximal lead. With this arrangement the electrical "artefact" appeared with weaker shocks than usual. Figure 13 shows the disturbances evoked with coil distances at which break shocks amounted to 147 Z (in *A*) and 372 Z (in *B*). When the stimulating electrodes were placed 55 mm. away from the proximal lead no excursions of the string resulted from these shocks; stronger shocks were not tried.

The nerve which was killed by immersion in hot Ringer solution yielded with powerful shocks very small excursions similar to those in figure 13, *A*, but opposite in direction.



A B

Fig. 12. Transient effects in dead nerve (see text). String E. Tension the same as in figure 11. Low-voltage magnet coil; core not grounded. Galvanometer leads 25 mm. apart on the nerve. Experiment 19. Cu and Hg key.

It is interesting to note that in these experiments with non-functional nerves the current through the string is in the same direction as in the case of the blank resistance, except in the case of the nerve killed in hot Ringer. It should also be noted that a small notch in the record from a make shock taken from this nerve just before it was heated, shows a transient current in the same direction as in the make shocks with the blank resistance and with the other inactive nerves. There appears to have been a reversal of direction in the transient current correlated with heating the nerve. A possible explanation might lie in the reduction of capacity which is a very probable consequence of destroying the nerve structure with heat. If the nerve's capacity were so reduced that the greater part of the capacity of the entire system was in the magnet core, we should expect such a reversal of the tran-

sient current through the string. The length of nerve beyond the distal lead was not recorded in these experiments as its possible significance had not become apparent to us at the time the experiments were performed.

In all these cases of transient current arising from induction shocks in which we used inert conductors, both in the case of the blank resistance and in that of the inactive nerve, the excursions are of a simple character. It is difficult to see how some of the more complex curves we have recorded from active nerves stimulated with powerful shocks can be interpreted as compounds of the simple electrical "artefact" and the equally simple monophasic action current. In attempting to determine as far as possible the nature of the disturbances, we made use of the latencies under various conditions of stimulation. Meas-

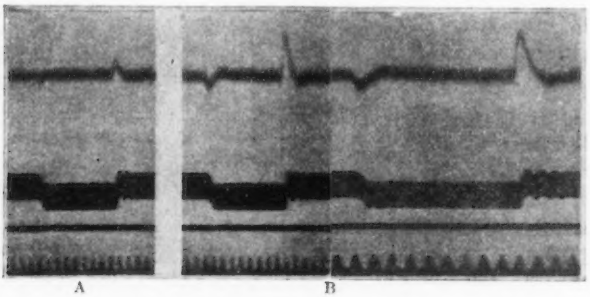


Fig. 13. Inactive nerve (peroneal), see text. String C. High-voltage magnet coil. Both speeds of film shown in B, procedure otherwise identical in these two pairs of shocks. Experiment 7. Knife-blade key.

urements were made on the films of the time interval between the first motion of the signal magnet and the first deflection of the string. In such measurements our accuracy was limited by the degree of accuracy with which time could be measured (readings being possible only to within about 0.4σ of the true value) and by the slight lag of the signal magnet which, as stated in our previous paper, appeared to vary slightly and to have a mean value of about 0.6σ. Considering the shortness of latency in recording the response of a nerve to direct stimulation, the percentage error is necessarily large. Consistent and significant results were, however, obtained from these measurements. Submaximal stimuli (ranging from 7 to 40 Z) give latencies varying according to the distance from the stimulating electrodes to the proxi-

mal lead and indicating a velocity of the nerve impulse in the neighborhood of 20 or 30 meters per second. For instance, in Experiment 28, with a distance of 50 mm. a submaximal response followed a latency of about 2σ. In general, when the stimuli were increased greatly in strength the latency became clearly less; in some preparations a diminution was evident with shocks of less than 100 Z, in others it was not apparent until a value of about 200 Z was reached. With shocks amounting to several hundred Z units the latency was reduced to within the limits of observational error. Whenever a preliminary notch was present which was clearly distinguishable from the action current, it was so nearly simultaneous with the first motion of the signal magnet that no interval could be detected with certainty.

A transient current passing through the string in the manner just analyzed and attaining sufficient magnitude to produce an appreciable excursion of the string, would naturally deform the curve in the resulting record, and since it necessarily occurs practically at the instant of the break of the current, such deformation will precede the part of the curve due to the action current proper. In those cases in which no such clearly defined notch occurred and in which, nevertheless, the latency was clearly diminished, the question arises whether the first excursion is due to any such electrical "artefact" or to a true action current appearing after a briefer interval from the stimulus than usual. Many records of action currents with shortened latency but showing no deformation whatever and produced with stimuli between 100 and 200 Z units lead us to suppose that the latter is the case, that we are dealing with an unusually prompt action current and not an "artefact." Such unusual brevity of the latency does not necessarily imply an unusually high velocity of the nerve impulse, for it can be explained as a result of the spread of electrical disturbance along the nerve trunk. Referring again to figure 7 it will be seen that there is a general convergence of lines of current flow toward the stimulating electrode not only from the portions of the nerve lying in the direction of the other terminal of the inductorium but also from all other portions of the nerve in proportion to their capacity. As these lines of transient current flow traverse the individual fibers there will be an infinite series of physiological anodes and kathodes. If the shock be strong enough, physiological kathodes, at which the current flow becomes sufficiently intense to stimulate the fibers, will be found at a considerable distance along the nerve from the stimulating electrode. If this is the case, excitation will occur at these points at the instant of stimulation and

the impulse will have less distance to travel before reaching the proximal lead. In this way the observed shortening of latency may readily be explained.

b. The effect of the signal magnet. Certain observations in other experiments with the apparatus revealed the possibility of transient currents resulting in part from the arrangement of our signal magnet. As was stated in our previous paper the wires to the signal magnet in the primary circuit of the inductorium were enclosed in lead sheathing and the lead put to earth. This introduced an appreciable capacity in the primary circuit. It is as if the primary circuit were connected with one plate of a condenser of which the other plate is put to earth. To determine what part this might play in the deformation of the records powerful shocks, with the secondary coil at zero, were used to stimulate a nerve, the signal magnet being included in the primary circuit as usual, and the responses to both make and break shocks were recorded. Then the signal magnet was disconnected from the primary circuit and in its place was introduced an approximately equal resistance in order to keep the primary current the same. Again make and break shocks, being of substantially the same intensity, were recorded. The curves traced by the string were almost identical in the two conditions. To render this evident we have reproduced the records together (fig. 14) by superposing the films and printing through both. Evidently the signal magnet with the capacity involved contributes practically nothing to the deformation of the action current records caused by powerful shocks.

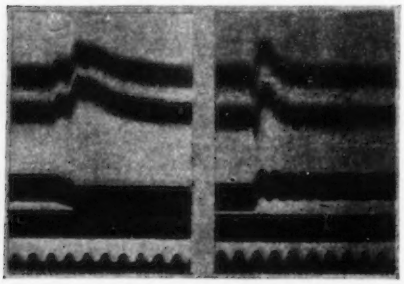


Fig. 14. Showing removal of signal magnet (see text). The lower galvanometer curve in each is the one made without the signal magnet. With signal, primary current 0.280 amp., break shock 2020 Z; without signal, 0.290 amp., 2080 Z. Experiment 9. (The curves made with the signal are duplicates of those in figure 3, C 5.) Knife-blade key.

c. Reversal of the direction of shock. In two experiments the effect of reversing the wires from the secondary coil to the stimulating electrodes was examined. The results of these experiments are shown in figure 15. In each case the secondary coil was at zero. In the upper

row (showing an experiment with coil *F*) the value of the break shocks was about 2000 Z units. Figure 15, *A*, shows both make and break shocks with the usual wiring, the kathode on the break shock being nearest the galvanometer. *B* shows similar shocks in the reverse directions. The lower row shows an experiment with a short coil (not

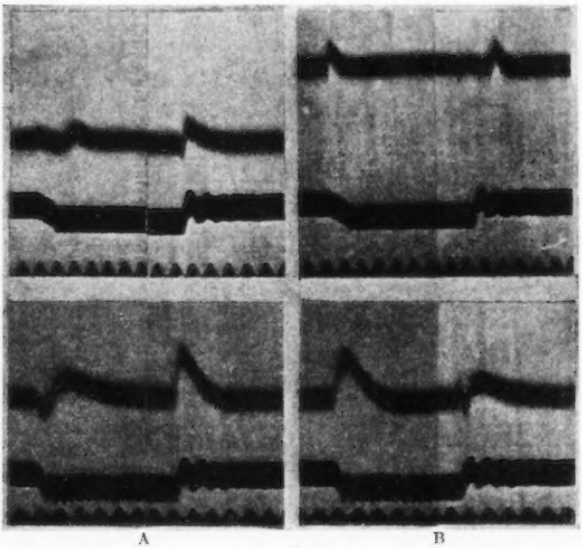


Fig. 15. Description in text. String D. High-voltage magnet coil. Upper row, Experiment 31. Sciatic nerve, the same one as in figure 4, B, after being kept over night (14 hours) in Ringer solution at room temperature. Break shocks each 1990 Z. Knife-blade key. The overnight impairment may be seen by comparing the make and break in B with those in figure 4, B 4, in which the shocks are the same; the nerve was replaced in as nearly as possible the same position on the electrodes in the morning as on the night before. Lower row, Experiment 14. Cu and Hg key. The insulation at one point in the magnet coil having burned out just before this experiment, it was only possible to use the 110-volt current, instead of the usual 220-volt current, to excite the galvanometer magnet (cf. previous paper, p. 138, legend to fig. 6).

calibrated) whose polarity had not been determined. It will be seen that the shapes of the curves are similar when the shocks are in the same direction regardless of whether they are make shocks or break shocks; yet appreciable differences exist between "ascending" make

and "ascending" break and between "descending" make and "descending" break.[28]

d. The influence of the iron core. A few attempts were made to see what effect the presence of an iron core in the inductorium might have on the nature of the deformation of action current records. To this end, since coil *F* was so constructed that the core could not be removed, another having a movable core, was employed. This coil, which we

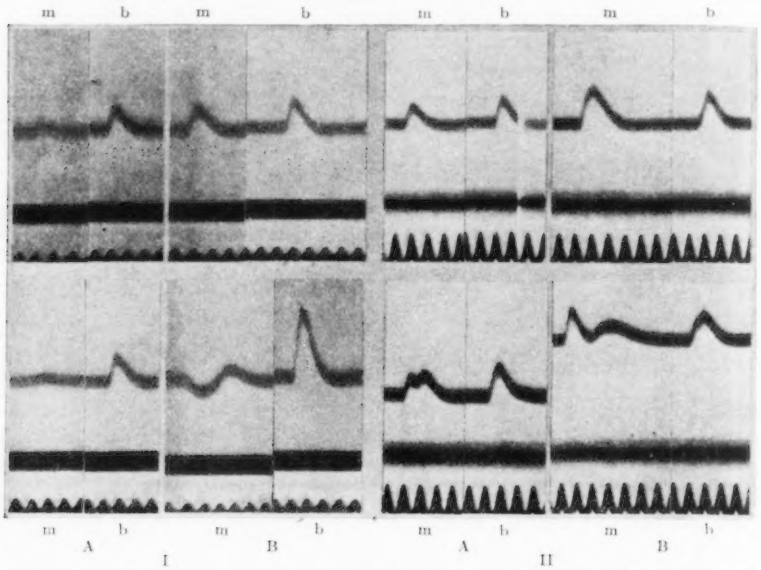


Fig. 16. Influence of iron core. Coil *X*, polarity not determined. Cu and Hg key. String C. Low-voltage magnet coil. Core not grounded. Primary current in both experiments about 1.5 amp. Lower row, iron core in coil; upper row, core removed. Sciatic nerve used in each; and placed in moist chamber. I, Experiment 27. II, Experiment 28. Differences described in text.

In both I and II, A was made with coil distance, 95 mm., B, coil distance, 0 mm. Approximate strengths of break shocks as follows:

Upper row: A, 104 Z; B, 2200 Z. Lower row: A, 915 Z; B, 7800 Z.

designate coil *X*, is short, the primary and secondary each being only about 7 cm. long, and is provided with bundles of soft iron rods tied together to make a compact and readily movable core. Comparative series of stimuli were studied with this coil both with and without the

[28] Cf. figure 4, lower row and explanation in legend.

iron core and the results were compared with those of stimuli derived from coil *F* and applied to the same preparation. A rough calibration of coil *X* was made, both with and without the core, by comparing the primary currents required to give threshold stimulation to a frog's muscle at different coil distances, and standardizing the values with a coil previously calibrated by Dr. Martin. Without taking the time for a very careful calibration of this coil we were still able to obtain a fair idea of the approximate value of our stimuli as judged by the criterion of thresholds.

Figure 16 shows the results of two experiments with different preparations but with substantially the same procedure. The only differences noted were that in Experiment 28 (second half of fig. 16) the nerve was in better condition than in Experiment 27, and the whole preparation (electrodes and nerve) had a lower resistance, 18,000 ohms as compared with 50,000. It is probable that both the nerve and the porous "boot" electrodes were drier in Experiment 27. In Experiment 27 the stimulus was applied 36 mm., from the proximal lead, while in this part of Experiment 28 it was applied 50 mm., from the proximal lead. In all other respects as far as details were noted the procedure was the same. In the upper row are shown the records obtained with coil *X* without the iron core. Immediately below each of these is the record obtained under identical conditions and with the same position of the secondary coil, but with the iron core. It should be noted that only one record in the case of Experiment 27 shows deformation, while with shocks of precisely the same strength in Experiment 28 all but one show notable deformation. This difference may be explained by the greater dryness of the nerve and the higher resistance in the circuit. It is also notable that the deformations in the two experiments are quite different in character.

It appears from a comparison of these records that the deformation, especially on make shocks, is much more marked when the core is present than when it is not. But it must be remembered that the shocks are (as indicated in the legends of the figures) far more powerful, other things equal, when the core is present, and that therefore the increase in deformation might be the result of increased intensity and not due to any peculiar effect of the core as such. To make a valid comparison it is desirable to use shocks of as nearly as possible the same intensity, offsetting the intensification due to the core by either weakening the primary current or by increasing the coil distance. No strict comparison of this sort was made with coil *X* but it may be

noted that in Experiment 28 (fig. 16) the deformation on the make shock is far more marked with the core in and a coil distance of 95 mm., than with the core out and the secondary coil at zero, while the difference between break shocks under corresponding conditions is comparatively slight. We have not determined the necessary constant "C" for evaluating make shocks, but the approximate values of the break shocks in the two cases are 915 Z and 2180 Z respectively, much greater in the arrangement with which the made shock produces the smaller deformation. Thus records tend to show that the iron core is an important factor in the causation of the more elaborate deformations. Figure 17 shows a pair of shocks, make and break, from coil *F* in Experiment 28, recorded immediately before those from coil *X* shown in figure 16 *II*. The break shock here amounts to 2080 Z which is, within the limits of accuracy in our calibration, equal to the break shock produced by coil *X* with the core out and the secondary at zero (fig. 16, *II B*, upper row). Here again the make shocks differ greatly, but we have not the data for estimating their relative values by the criterion of thresholds.

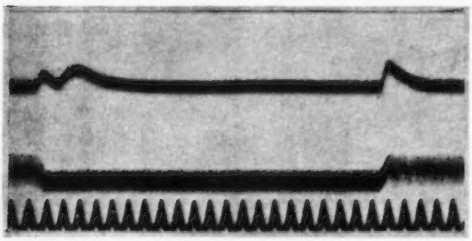


Fig. 17. Experiment 28. Coil *F*. (See text.) Primary current, 0.285 amp. Break shock, 2080 Z. Usual wiring and direction of shock. Cu and Hg key. Compare with figure 16, II.

Figure 18 shows two series of records also bearing on the influence of the iron core. They were obtained with corresponding shocks in the two parts of Experiment 28, the stimulating electrodes being 50 mm. and 27 mm. respectively from the proximal leads. Here, as in other experiments, it is evident that the deformation becomes more marked when the stimulating electrodes are near than when remote from the leads.

It is noteworthy that the highly complex curves resulting from powerful shocks are only obtained when an iron core is present in the coil. The most powerful shocks obtained without the core, even when applied close to the leads, produce only a bending of the first limb of the curve (away from the base line on the make, toward it on the break) such as we might expect from a simple transient current practically synchronous with the action current. It seems, then, that the peculiar

phenomena denoted by the complex curves shown are in some way associated with the iron core of the inductorium.

e. Double or compound stimulation. We have already noted that the deformation in some of our records suggests as its cause a second impulse in the nerve, and that Garten construed a similar observation in this way.[29] In order to consider properly the theoretical possibility of such an occurrence we must refer to Nernst's theory of electrical

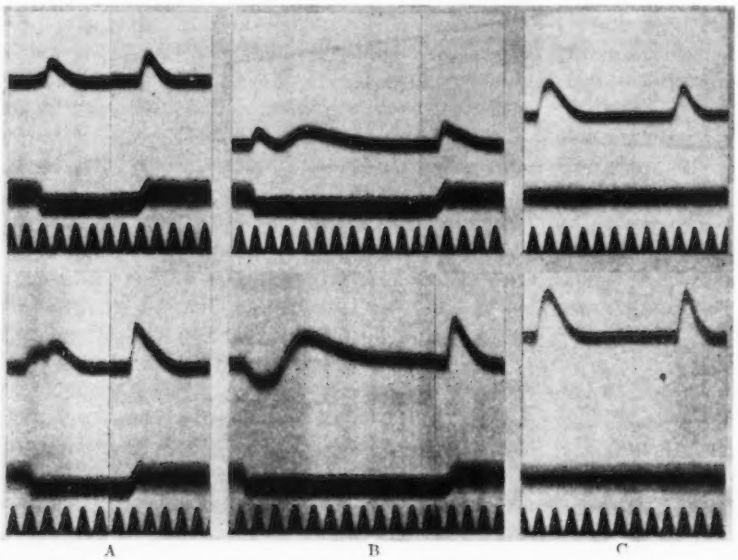


Fig. 18. Experiment 28, upper row, stimulus 50 mm. from proximal lead; lower row, stimulus 27 mm., from proximal lead.

In both rows: A and B, Coil *F*. Primary current, 0.49 amp. C, Coil *X*. Primary current about 1.5 amp.; no iron core.

A, coil distance, 123 mm., break shock, 1400 Z; B, coil distance, 0 mm., break shock, 3700 Z; C, coil distance, 0 mm., break shock, 2200 Z.

In C, the records are continuous between make and break shocks. In all Cu and Hg key.

excitation which has been modified by Hill,[30] and which in its modified form has been shown by Lucas[31] to afford a most satisfactory basis for

[29] Garten: Loc. cit.

[30] Hill: Journ. Physiol., 1910, xl, 190.

[31] Lucas: Journ. Physiol., 1910, xl, 225.

interpreting the observed relations between the strength and duration of current required to excite various tissues. As summarized by Lucas,[32] this theory is based on the assumption that an exciting current produces its effect by concentrating certain of the ions by which it is carried at membranes (impermeable to the ions) contained within the excitable cells. In his modification of the theory Hill, in order to explain the relative inefficacy as stimuli of slowly increasing currents, has further assumed that the propagated disturbance is initiated by the breakdown of some unstable substance in the cell, normally in equilibrium, but acted on by the ions involved in excitation when these are more concentrated than normal. He assumes that when the ions are sufficiently concentrated (the requisite degree of concentration varying with the amount of unstable substance present) the reaction proceeds explosively, much as hydrogen and oxygen combine when heated together above a certain temperature.

The theory avowedly deals only with the conditions necessary at the point of stimulation for starting the propagated disturbance, and does not attempt to explain the nature of the latter. It gives us no clew to the refractory phase or the related fact that excitable tissues respond rhythmically and not continuously to continuous stimuli. It has been shown by Garten[33] that a constant current applied to a motor nerve can produce a rhythmic response in the nerve or in the innervated muscle, in fact, the "kathodal closing tetanus" is a classical observation.[34] It is also a familiar fact that the amphibian heart, brought to a standstill by a Stannius ligature, will respond with rhythmic contractions to continuous stimulation either with a rapid series of induction shocks or with a constant current. Just how recovery from a preceding response occurs and why the continuous stimulus produces rhythmic response are matters which appear not to have been explained. Following Hill's hypothesis we may suppose that recovery depends on the renewal of the unstable substance. But how this substance is protected by a refractory period from the action of the ions and permitted to accumulate till it reacts again in an explosive manner, instead of being acted on continuously as long as the ionic concentration is maintained by the current, is not evident. As has already been indicated by one of us [35] in connection with the activity of certain reflex centers, this

[32] Loc. cit., 227.

[33] Garten: Loc. cit., 557.

[34] See Howell: Text Book of Physiology, Fourth Edition, 1911, 91; Schafer: Text Book of Physiology, 1900, ii, 510.

[35] Forbes: Proc. Roy. Soc. B., 1912, lxxxv, 298.

tendency to rhythmic response denotes some peculiarity in the relation between the substances involved in the processes of excitation, response and recovery not covered by the conception of an unstable substance reacting with ions in accordance with the mass law.

At all events, it is a salient fact in connection with excitable tissues that they may respond to continuous stimulation discontinuously, i.e., with a succession of separate propagated disturbances. Expressing it in terms of Hill's hypothesis, if when the unstable substance is renewed the effective ions are still maintained at a supernormal concentration by the current flow, a fresh disturbance will be initiated. The bearing of this on the effect of the powerful induction shocks which we have been examining is that if the ionic disturbance be great enough and the shock persist long enough with an intensity sufficient to maintain adequately the ionic concentration, the refractory period may end (i.e., the unstable substance be renewed) in time for a second disturbance to be initiated. Erlanger and Garrey[36] have shown that induction shocks persist longer than is frequently supposed, and as outlined above it is only necessary that when the refractory period is over, the shock shall not have declined below the intensity required to maintain an effective concentration of ions in order that a second impulse shall be evoked. Judging by the refractory period shown by Adrian and Lucas[37] for amphibian nerve, and the temperature coefficient of the same shown by Gotch and Burch[38] a powerful break shock may well maintain a local excitatory process long enough to initiate two or even three impulses in a mammalian nerve, even though the local excitatory process outlasts the exciting current by a negligibly short time.

The fact that some of our electrical records produced with powerful shocks show only such deformation as we should expect to result from a second impulse, taken in connection with the theoretic considerations just discussed, makes it seem not improbable that a second impulse may result from shocks of great intensity. In this connection it may be of great significance that make shocks have been found by Erlanger and Garrey to subside far more gradually than break shocks. For in our records, as has already been noted, the deformation is far more marked in the case of make shocks than in that of break shocks under similar conditions, although the break shocks are of far greater stimulating intensity. The explanation may be that break shocks, intense

[36] Erlanger and Garrey: This Journal, 1914, xxxv, 403.
[37] Adrian and Lucas: Journ. Physiol., 1912, xliv, 114.
[38] Gotch and Burch: Journ. Physiol., 1899, xxiv, 416.

as they are, subside so quickly that they only produce one or at most two impulses, while the make shocks, persisting longer, may inaugurate a series of impulses comparable to the closing tetanus.[39]

We do not feel that this view can be confirmed with the data at hand, nor do we believe that the series of impulses can explain all the peculiarities of our more complex records not covered by the transient current already discussed. Still, we feel that these suggestions may constitute a step toward interpreting these rather baffling observations.

We have discussed the deformations at some length (1) because it seems important to emphasize the possible confusion in interpreting action current records due to the entrance of an "artefact," (2) because the electrical disturbance which causes the artefact in the record may have some physiological import depending on the occurrence of stimulation some distance from the physical electrodes, and (3) because the doubling or compounding of the stimulus, which we consider a likely consequence of powerful shocks, has probably an important bearing on the reflex responses, as will later appear.

In dealing with such powerful shocks as those we have been discussing, which produce deformed electrical records, and which may reasonably be supposed to evoke a succession of two or more impulses in the nerve fiber, the problem of evaluating shocks becomes too complex to admit of simple quantitative comparison. Any attempt to compare quantitatively the physiological values of shocks of different contour, as for example the make and break shocks of a given coil, must be made with due regard to its limitations. The rate of subsidence of a shock and of the resultant local excitatory process might well determine the stage of recovery from the first refractory phase at which a second impulse would be initiated. In other words, because, from a comparison which is valid in dealing with thresholds, shocks of different contour would appear to be equal in strength, it does not follow that their physiological effects are inevitably identical; if strong enough to be compound instead of simple stimuli their physiological effects may be quite different.

f. Résumé. Before proceeding to consider the gradation of reflex

[39] It should be noted, on the other hand, that we have not recorded many powerful make and break shocks with the secondary wires reversed, and in the cases in which we did (shown in fig. 15) the character of the response was shown to be in the main reversed. Figure 4, B3 shows suggestive results of make and break shocks with wiring reversed from the usual arrangement, illustrating the two types of deformation in their simplest forms.

responses to stimuli of graded intensity it will be advantageous to summarize briefly our findings with responses from the nerve trunk under direct stimulation. Our observations confirm the statement of Gotch that "the response of nerve passes through a series of submaximal stages of increasing value until a maximum is reached." They show that in the case of uninjured mammalian nerves such as we have studied this maximum is reached with break shocks of about 40 Z units, and that with stimuli of several times this value no further increase in the action current is recorded. We find that with more powerful shocks (the requisite strength seems to vary, dependent partly on the distance of the stimulating electrodes from the leads) the galvanometer no longer records simple action currents; the curves show deformation which in some cases appears to result simply from a transient current caused by the connection of the galvanometer with the inductorium and dependent partly upon the distribution of electrostatic capacity in the system (to which the nerve itself must contribute appreciably), which in some cases suggests a succession of nerve impulses, and which in others appear too complex to admit of interpretation with the data at hand.

C. Gradation in the reflex response

a. Electrical. Having dealt with the gradation of action shocks in nerve trunks in response to induction shocks of graded intensity applied directly, and in particular to the gradation of response in the afferent nerve used for reflex stimulation in our experiments, we may proceed to consider the gradation of reflex response resulting from similarly graded shocks. In several experiments measurements were made of the recorded excursions of the string obtained from monophasic action currents in the motor nerve resulting from the usual reflex stimulation. It has been noted in our preceding paper[40] that even with stimuli of constant strength and though a rest is allowed before each observation, the magnitude of the reflex electrical response is not always constant, but that a progressive increase commonly occurs, and in addition irrregularities without apparent cause are often seen.[41] In view of this fact and of the smallness of the reflex responses (no excursions amounting to more than 3 mm.) it was not possible to examine the gradation of reflex responses with anything like the accuracy ob-

[40] Forbes and Gregg: Loc. cit., 170, 173.

[41] Cf. Sherrington and Sowton: Journ. Physiol., 1915, xlix, 335.

tainable in the case of responses to direct stimulation. We could not determine with certainty whether gradation of reflex response occurred through a given series of stimuli except in those near threshold value. From such measurements as were made it appeared that the response increased from threshold to a maximum which was reached in some preparations at about 25 Z and in some not below 60 Z. After this maximum was reached the responses seemed to remain fairly constant in size and shape even after the stimuli were increased to several hundred Z units. But inasmuch as this was necessarily only a rough estimate, we cannot say with any certainty at what strength of stimulus gradation of response ceases, or indeed whether it really ceases at all.

b. Myographic. Although measurements of the string excursions yielded little information as to the gradation of reflex responses we were able to learn much from observations on the muscular contractions. We made significant observations in many experiments by inspection, and in two we made myographic records of muscular contraction on a smoked drum. In one of these (No. 21) a thread was attached to the ankle and so arranged that flexion of hip and knee should produce a rise in the myograph line. After a series of flexion reflexes had been thus recorded the thread and lever were so arranged as to record the crossed extension reflex, i.e., knee extension in the opposite leg. After a brief series of these the afferent nerve (popliteal) was removed and its action currents were recorded as usual. In the other experiment (No. 29) in which this method was used the muscles of the leg were all paralyzed by section of their nerves except the vasto-crureus and the knee flexor semitendinosus. The tendon of the latter was severed from its insertion and to it was tied a thread connecting it with the myograph lever. In this case the action currents of the afferent nerve were not recorded.

In Experiment 21 there was a progressive diminution in the magnitude of contraction with a given strength of stimulus throughout the experiment, probably due to local impairment of the afferent nerve in the stimulated region.[42] On this account it was necessary to compare stimuli in alteration. The results of the first part of the experiment in which the flexion reflex was recorded were as follows: A break shock of 68 Z units produced only a small contraction, 82 Z produced a clearly larger one. Satisfactory comparisons were not made between these values and larger ones, but a number of stronger stimuli were compared

[42] This would mean that stimuli late in the series had less physiological value than appears from the figures assigned them.

with each other. A marked increment occurred in the contraction on passing from 130 Z to 230 Z, 460 Z produced a notable further increment, and 1360 Z produced a marked increment over the latter. It should be noted that none of these flexor contractions obtained from single shocks, even when they amounted to over 2000 Z, were maximal; a series of six or eight make and break shocks of moderate intensity (the breaks being 100 Z) delivered in quick succession, i.e., in the course of one or two seconds, produced almost three times as large an excursion of the myograph lever as the largest produced by a single shock.[43]

In the series of records of the crossed extension reflex only powerful shocks were used and these were of three values, 1660, 1920 and 2180 Z units. Contractions resulted from single shocks and these were unquestionably graded in magnitude, an increment occurring with each increase in stimulus.

When the afferent nerve used in this experiment was removed and its action currents were studied it showed a graded correlation between response and stimulus of the sort already described as occurring in the majority of experiments (see p. 184, also fig. 1, lowest curve) and taken to signify local impairment of the nerve. An approximate maximum of response was reached at 83 Z, but slight increment persisted well into the hundreds. Moreover with the strongest stimuli the excursions were smaller than are commonly found when nerves in good condition are maximally stimulated. It seems probable that local impairment was such as to render some fibers wholly inexcitable. This state of affairs in the afferent nerve modifies the significance of the results, for instead of a definite limiting maximal value of response reached with stimuli of 40 or 50 Z units we have a persistent gradation of response, and with this we should expect a persistent gradation of reflex response which we might expect to be absent under more normal conditions.

In the other experiment (No. 29) with the myograph, in which the isolated semitendinosus was used as an indicator, the results were somewhat puzzling. Certain irregularities were found, but some facts in regard to gradation stood out clearly. The response to 174 Z showed an increment over the response to 135 Z, and 1420 Z produced a marked increment over 930 Z as is shown in figure 19; 1560 Z produced a further

[43] It is interesting to contrast this mechanical summation with the diminution of electrical effect regularly noted in similar series of stimuli in our previous paper, loc. cit., 168.

increment. No observations were made of the action currents in the afferent nerve in this experiment, and this fact prevents direct comparison of reflex with direct stimulation; but the experiment shows in general over how wide a range of intensities in stimuli gradation of response can occur.

c. Inspection. The most significant information was obtained by inspection of muscular movements; and in most cases this was later correlated with the findings from recording the action currents in the afferent nerve. It was readily possible to compare in alternation two or three different intensities of stimulus and to note whether the muscular activity was correspondingly graded.

The type of muscular response to single shocks varied greatly among the different preparations. Sometimes the contractions were only seen in the stimulated leg until shocks of great intensity were used. Sometimes the crossed extension reflex was almost as readily produced as the flexion reflex; in one experiment (No. 15) it followed single shocks of only 6.8 Z units. In the spread of reflex activity to muscle groups more or less remote from the stimulated limb we found the most striking evidence of graded correlation between central effect and stimulus. These muscle groups included those of all four limbs, the trunk, neck and tail.[44] It may be stated in a general way that consistent increments in reflex spread were found in the comparison of stimuli of widely varying strength. In some cases increase in the spread of reflexes resulted from increasing the strength of single shocks through the same range over which the response of the nerve trunk directly stimulated shows marked gradation, i.e., from

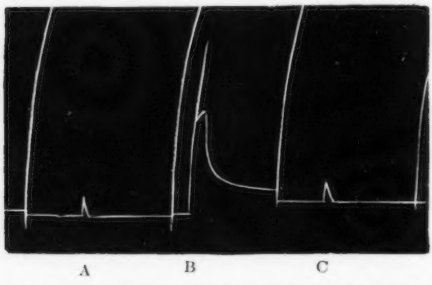


Fig. 19. Contractions of semi-tendinosus muscle in decerebrate preparation. Stimulus applied to peroneal nerve. Experiment 29. Stimuli, single break shocks: A and C, 915 Z; B, 1420 Z. Speed of drum, 5 mm. per second; ordinate lines indicate rests during which the drum was stopped.

[44] Cf. E. L. Porter: This Journal, 1915, xxxvi, 172. It is noteworthy that with single shocks we sometimes evoked forelimb extension, in one preparation with less than 100 Z, whereas Porter never obtained this response in the undrugged animal.

about 10 Z to 40 or 50 Z. In other cases no spread of reflexes occurred with stimuli of less than 100 or 200 Z. The most salient fact was that in practically every experiment gradation in the spread of reflex response followed the grading of stimuli into the hundreds, and when tested, into the thousands of Z units. In most cases large increments of stimulus produced only small increments of response, and in some cases increments of considerable magnitude (e.g., from 93 Z to 120 Z) produced no clearly marked increment of response. On the other hand, in one instance (Experiment 15) an increment in the spread of reflex response appeared with every increment in the following graded series of stimuli: 6.8 Z, 7.4 Z, 17.4 Z, 25 Z, 73 Z, 93 Z, 120 Z, 164 Z, 236 Z, 333 Z, 493 Z. We found regularly that increased spread followed any such increase of stimulus as that from 600 Z to 1300 Z or from 1200 Z to 1700 Z. In one case 3700 Z produced clearly more activity than 2000 Z.

This persistent gradation of reflex response to single shocks is remarkable in view of the fact, noted earlier in the paper, that in the afferent nerve, if uninjured, gradation of response ceases at about 40 Z units, all responses to stronger stimuli being maximal. There appears to be here a striking paradox. The nerve trunk stimulated with a shock of 60 or 100 Z is apparently conducting as great a physiological disturbance as it is capable of, and yet when stimulated with a shock of 200 Z it evokes a greater disturbance in the central nervous system than when stimulated with only 100 Z; with 500 Z a greater response is evoked than with 200 Z, and with 1000 Z a greater response yet. By what additional activity in the afferent nerve trunk is this additional central activity evoked?

Inasmuch as most nerve trunks whose action currents were studied showed gradation of response as far as the responses were capable of valid comparison, probably in consequence of local impairment, it might be supposed that this same local impairment was likewise responsible for the persistent gradation in the spread of reflexes. This may be so in many cases, and yet it is fair to assume that in every case the afferent nerve was far less impaired during reflex stimulation than it was when its action currents were subsequently recorded. For, in the first place, reflex stimulation was always carried out before the recording of action currents, and consequently while the nerve was fresher; and, in the second place, the nerve was always protected during reflex stimulation by the glass tubing of the Sherrington shielded electrodes and kept covered by the muscles which naturally surround it;

in the subsequent removal from the animal's body it was liable to greater impairment than in this first part of the experiment. But even so, we cannot be certain without direct evidence that in the reflex part of the experiment there has not been enough local impairment to render certain fibers inexcitable except to the most powerful shocks, and thus to admit of explanation of the reflex gradation on the same terms as the direct gradation. It is necessary to ascertain whether in a single experiment gradation can occur in the reflex response to stimuli which are subsequently shown by the direct recording of action currents to produce only maximal responses in the stimulated nerve.

Experiment 15, just cited as showing reflex gradation over an exceptionally wide range of stimuli, is of interest in this connection. Local impairment here must have been slight and one of the other progressive factors mentioned as tending to increase the galvanometer excursions must have played a part, for the excursions of the string increased progressively throughout the series of recorded action currents when stimuli of the same strength were repeated. This increase was so marked as to render difficult the comparison of different stimuli. It is nevertheless possible to state that very little true gradation in the action current occurred with stimuli over 30 Z, and that above 60 Z such gradation as may have occurred fell within the limits of observational error, the increase amounting to not more than 2 per cent between 60 Z and 164 Z, above which value the responses showed deformation. It will be recalled that in this experiment gradation of reflex effect was found continuously from 6.8 Z to 493 Z, and in particular at the successive intervals between 73 Z, 93 Z, 120 Z and 164 Z, over which range of stimuli gradation was virtually absent from the responses of the nerve itself.

A clearer and more satisfactory result was obtained from Experiment 21 B. In this case the whole sciatic nerve, examined when still fresh, yielded at 42 Z a clear action current maximum which was sustained to the point of deformation as shown in the uppermost curve of figure 1. Unfortunately, reflex effects of stimuli ranging between 50 and 150 Z were not compared with each other, but a clear increment of response was found with 230 Z over that obtained with 83 Z, and an increment marked each of the following over its predecessor: 230 Z, 330 Z, 467 Z, further increments appearing on passing from 905 to 1160 Z, from 1160 to 1950 Z and from 1690 to 2200 Z. Here, then, is a case in which the afferent nerve was apparently maximally stimulated by every shock of more than 42 Z, and yet successive increments in the disturbance

evoked through it in the central nervous system could be regularly shown to follow every substantial increase in the intensity of the induction shock from this value to over 2000 Z units. The recent experiments of Sherrington and Sowton, already cited, seem to point in the same direction, although their stimuli were not measured in a scale of units which admits of comparison with ours.

It is clear, then, that after induction shocks have been increased in intensity to the point at which, judged by the action currents, they produce maximal stimulation of an afferent nerve trunk, their further increase can in some way so affect the nerve as to cause it to induce in the reflex centers greater and greater activity. In this connection, the observations of Martin and his co-workers should be reviewed. Martin and Lacey[45] found that in the decerebrate cat induction shocks delivered to any large afferent nerve at rates of from 2 to 60 per second if of moderate intensity produce depressor effects on blood pressure, and that "to obtain pressor reflexes stimuli well in excess of 250 Z units must usually be employed." The average threshold for pressor effects in their series was 280 Z. Martin and Stiles[46] found that stimulation of the central end of the cut vagus, under somewhat similar conditions, produced two types of depressor effect, the threshold for the first being about 10 Z, and the threshold for the second being about 200 Z or higher (roughly of the same order as the pressor threshold in the case of the stimulation of other afferent nerves). In a later paper Stiles and Martin[47] remark of the pressor reflex from stimulating afferent nerves in general that with additional increase of stimulation (above 280 Z) "the elevation of the blood pressure becomes more and more marked through a long range." In these experiments, especially those in which stimuli were applied to the same nerves that we have used, it is probable that examination of the action currents in the stimulated nerves would have shown limiting maximal values in the vicinity of 40 or 50 Z units, inasmuch as the type of electrodes and other significant experimental conditions closely resembled ours. The observations of these workers appear, therefore, to confirm the fact that although an afferent nerve is maximally stimulated (judging from its electrical response) it may not evoke as great a central disturbance as it would if stimulated with more powerful shocks.

A confusing factor might have to be reckoned with when shocks are

[45] Martin and Lacey: This Journal, 1914, xxxiii, 212.
[46] Martin and Stiles: This Journal, 1914, xxxiv, 106.
[47] Stiles and Martin: This Journal, 1915, xxxvii, 95.

applied in rapid succession as was the case in the researches just mentioned, for Adrian and Lucas[48] have shown that following the refractory period in a stimulated tissue there is first a stage of gradual return to normal conductivity and then a stage of slightly supernormal conductivity. The entire series of changes in amphibian nerve at about 15°C. only lasts at most 0.1 second, and probably much less in mammalian nerves at body temperature. The phase of heightened conductivity could scarcely affect the present case unless there has been profound impairment in the nerve trunk, for we are dealing with supra maximal stimuli; all the fibers are presumably adequately stimulated and conducting impulses with all strengths of stimulus under consideration. The "relative refractory period," during which excitability and conductivity are subnormal, lasts in a nerve trunk at most 0.02 second, too short a time to play a part in the researches we are considering. For Martin and his co-workers found the gradations of response which we have mentioned irrespective of the frequency of stimulation between the limits of 2 to 60 per second. In short, each stimulus must have found the nerve completely recovered from the refractory period following its predecessor, and may, therefore, be considered in the present argument as a single shock. The summation of propagated disturbances which is well known to result in the central nervous system from such frequencies of stimulation as those just mentioned do not concern the issue under discussion.

E. L. Porter,[49] dealing with reflex effects identical with those which have been the basis of our observations, viz., the spinal limb-reflexes, found that in the spinal cat the threshold stimulus for the crossed-extension reflex varied all the way from 5.8 Z to 300 Z. For stimuli he used single break shocks, and all possible confusion from repetition of stimuli was therefore eliminated. His experimental conditions were closely comparable to ours. The fact stands out from our experiments and from those of others who have reckoned their stimuli quantitatively, that "supramaximal" stimuli are not all equal in their central effects, but that even among them gradation of strength causes corresponding gradation of reflex response.*

[48] Adrian and Lucas: Journ. Physiol., 1912, xliv, 106, etc.

[49] E. L. Porter: Loc. cit.

*Graham Brown (Proc. Roy. Soc. B. 1913, lxxxvii, 142), in obtaining his graded series of reflex contractions used coil distances down to 70 mm. He does not furnish the other data for the evaluation of his stimuli, but it is almost certain that the strongest amounted to several hundred Z units, and would not have yielded simple action current records in the afferent nerve.

D. Proposed explanation of reflex increment

The problem of explaining this fact we have been unable to solve to our satisfaction. Five conceivable explanations have occurred to us, and of these one has been definitely excluded by experiment. They are as follows:

1. Some of the fibers in the afferent nerve, either because of being relatively inaccessible to the stimulating current or of having much higher thresholds than the rest, are only excited by powerful shocks, and are either so few in number or so well insulated by surrounding fibers from the leading-off electrodes that their activity does not add appreciably to the action current as recorded in the galvanometer. We have found no way to test this possibility. If their failure to add appreciably to the recorded disturbance were due to their small number it would be surprising that their meager addition to the total number of impulses could induce in the centers an activity so enormously greater than is evoked by the great majority of the fibers acting without them as is seen to be the case. But if it could be shown that moderate induction shocks only stimulate the surface layers of fibers, leaving the majority of more protected fibers undisturbed, and that the galvanometer records the action currents of only the same surface layers of fibers, then in the stimulation of the inner, more protected fibers with powerful shocks we should have a plausible basis for the gradation of reflex response as we have seen it. Against the probability of this explanation we may mention the fact that we have regularly placed the stimulating electrodes obliquely on opposite sides of the nerve. Thus it is probable that the stimulating current has attained, even with moderate shocks, an effective density in the central as well as the superficial fibers.

2. It may be that the electrical disturbance is not a true quantitative criterion of functional activity. It is quite conceivable that the action current may reach a limiting maximal value and yet the physiological activity be capable of further intensification which reveals itself only indirectly. Lucas[50] has pointed out that the relation of the electric response to the propagated disturbance is unknown. Gotch[51] has argued that under certain special conditions a nerve impulse may occur without electrical concomitant. The possibility that the elec-

[50] Lucas: Proc. Roy. Soc. B, 1912, lxxxv, 502–508.

[51] Gotch and Burch: Journ. Physiol., 1899, xxiv, 426; Gotch: Journ. Physiol., 1902, xxviii, 51, etc.

trical response may be limited in magnitude while the propagated disturbance is not, or at least has a different limit, agrees with Gotch's contention as to their separability. Gotch's conclusion was based, first on the observation (made jointly with Burch)[52] that when two stimuli were applied with an appropriate time interval to a cooled portion of a nerve, only the first evoked clearly appreciable electrical disturbance in a capillary electrometer connected with the cool part, yet when the instrument was connected with a more remote warmed portion of the nerve two responses were recorded. This evidence was reinforced by his later observation that the electrical disturbance could not be detected in a nerve within 4 mm. of a recent injury, although a propagated disturbance could be evoked in and conducted through the same region.

Lucas[53] in 1912 in discussing the relation between action current and propagated disturbance, pointed out that Adrian's observations, then in progress (assuming they proved valid) showed how the observation of Gotch and Burch could be explained without the assumption of any lack of parallelism between action current and nerve impulse. Adrian[54] has since completed and published these experiments in which he showed that when a propagated disturbance passes through a region of impaired conductivity in which it undergoes a decrement, it will, if not wholly extinguished, regain its full magnitude (as judged by its ability to pass through another region of decrement) on emerging into a normal region. Taking this fact in connection with the phenomena of the relative refractory period, Lucas' explanation of the observation of Gotch and Burch becomes well founded.[55]

In like manner we may explain Gotch's other evidence,[56] the disappearance of the electrical effect in the neighborhood of an injury. Such a modified region may be one of impaired conductivity similar to that studied by Adrian. An impulse entering such a region might be so reduced as to be too small to produce a visible excursion in the electrometer; yet a disturbance initiated in this region might succeed in passing out of it without complete extinction till it reached the unaffected region where it could regain a normal magnitude. Gotch's observation that the neighborhood of a fresh injury manifests hyperexcitability

[52] Gotch and Burch: Loc. cit., 422.
[53] Lucas: Loc. cit., 505–506.
[54] Adrian: Journ. Physiol., 1912, xlv, 389.
[55] Cf. Boruttau: Pflüger's Arch., 1901, lxxxiv, 417–424.
[56] Gotch: Loc. cit., 54.

may seem to contradict this view of impaired conduction; yet this fact might be accounted for in the following way. We have frequently noted in our experiments that rapid severance of an afferent nerve trunk produces a more profound and persistent central effect than any single induction shock, and that crushing or ligature produces a more profound and persistent effect still. The former difference might be explained, if the strongest shocks failed to excite all the fibers, by the fact that section necessarily does excite them all, without the assumption of more than single maximal stimulus to each fiber; but the difference between section and crushing shows that in the latter there is something more than a single stimulus. In severe mechanical injury there appears to be a persistent source of stimulation. The way in which such a sustained stimulus would act will be discussed in a later section; but the bearing of it on the present question is that on the basis of Nernst's theory of excitation[57] a moderate degree of "local excitatory process" may be supposed to last for some time after an injury such as Gotch inflicted. If this were so, then even though the conductivity, and with it the ease of initiating the propagated disturbance, were notably impaired, hyperexcitability might yet be found.[58]

In view of these considerations and the close parallelism between the electrical disturbance and the "propagated disturbance" shown by Lucas,[59] we are disinclined to accept the view that functional and electrical disturbances are separable to the extent that great increments in the former can be induced by increasingly powerful single shocks after the latter have reached a limiting maximal value.

3. The third explanation was suggested by some of the observations recorded in the first part of the paper on transient electrical effects. We have shown (p. 189) that an appreciable transient current flows through remote portions of a nerve or other conductor similar in resistance and capacity when any part of it is subjected to a powerful induction shock. In our previous paper we mentioned the fact that even when recording the response in nerve to reflex stimulation a notch in the record denoted the passage of such a transient effect through the string.[60] These notches were not uncommon, and as might be

[57] See Hill: Journ. Physiol., 1910, xl, 190.

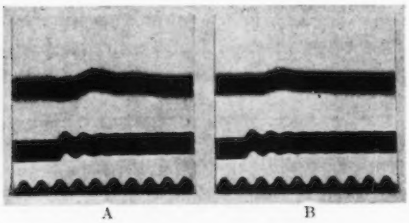
[58] For the differentiation of the stages in excitation, see Adrian and Lucas: Journ. Physiol., 1912, xliv, 69.

[59] Lucas. Journ. Physiol., 1999, xxxix, 207; cf. also Boruttau: Loc. cit., 325, etc.

[60] Forbes and Gregg: Loc. cit., p. 140; also figure 9.

expected were notably of more frequent occurrence in those few experiments in which, with the high voltage magnet, the distal nerve lead was connected with that end of the string which was put to earth through the magnet core than they were in those in which the reverse wiring was employed.[61] The difference is illustrated in figure 20, (*A*) being made with the former wiring and (*B*) with the latter. The direction of the current through the coils of the magnet was also reversed in order that the action current should be recorded by a deflection of the string in the usual direction.

The occurrence of a small excursion of the string at the instant of stimulation in these reflex experiments in which the nerve under observation is separate from that which is stimulated shows that from the stimulating electrode an appreciable electrical disturbance spreads not only along the nerve trunk but to some extent throughout the entire connected system of conductors, including the animal's body. In view of this fact, it occurred to us that the great spread of reflex activity to remote centers in the spinal cord resulting from powerful shocks might be due to the direct stimulation of the nerve centers by the transient current. It was an easy matter to test this experimentally, and in those preparations used for the test the result was so definite that only three or four experiments seemed necessary to settle the point. After a series of responses to various powerful shocks had been observed the afferent nerve was crushed at the hip without disturbance to the electrode contacts; a similar series of shocks was then repeated. In one case the muscles adjacent to the point where the stimulated nerve entered the cover of other tissues responded with slight twitches to the most powerful shocks (2000 Z). These were evidently the result of direct or motor nerve stimulation and not of reflex excitation. In



A B

Fig. 20. Experiment 18, reflex responses of peroneal nerve. String D. High-voltage magnet coil. (See text.) Stimulus, 40 Z in each. Cf. previous paper, figure 10, 2 A, and figure 11, 3 B, taken respectively before and after those shown here from the same preparation under practically the same conditions.

[61] See p. 190, and footnote 25; also p. 193, and figure 11.

the remaining cases no muscular contractions were produced by shocks of over 2000 Z even when repeated rapidly to induce, if possible, reflex summation. Clearly, such transient electric currents as traversed the body of the animal were inadequate to stimulate the reflex centers directly. This tentative explanation of the spread of reflexes must be abandoned.

4. Another possible explanation might be followed on the principle suggested by the shortened latency of the action currents of nerves with powerful shocks (p. 197). It will be recalled that the latency was appreciably shortened with shocks of about 100 or 200 Z, and practically disappeared with still stronger shocks. We pointed out how such shortening of latency might result from the spread of current along the nerve in sufficient concentration to stimulate some of the fibers at a considerable distance from the point where the stimulating electrodes are applied.[62] If this were so to a marked degree the impulses would be initiated in the various fibers at various distances from their central terminations, for with some variation in the thresholds of the individual fibers, and with some variation in the current density in different areas of the nerve's cross section there would be a wide distribution of the actual points of stimulation among the fibers. Some would perhaps only be stimulated immediately under the physical kathode, while in others an adequate physiological kathode would be found several centimeters nearer the center. In this way some impulses would have a head start over the others, and the effect in the center would be not a "volley" but a "platoon fire."[63] On reaching the central network they would traverse common paths not simultaneously, but in succession, and summation might conceivably result. It is a familiar fact that summation occurs to a marked extent in reflex arcs;[64] Adrian and Lucas have pointed out that it is a "summation of propagated disturbances."[65] They have suggested that reflex summation may depend only on the conditions which they have produced artificially in the nerve trunk, namely, a region of decrement (impaired conductivity) and the appropriate timing of successive impulses. They have shown that following the absolute refractory period the excitability and conductivity return gradually to normal, then for a time become supernor-

[62] Cf. Adrian: Journ. Physiol., 1914, xlvii, 473.

[63] Cf. Brucke: Sitzungsb. d. Wiener Akad., 1877; Buytendyk: Zeitschr. f. Biol., 1912, lix, 36.

[64] See Sherrington: Integrative Action of the Nervous System, 1906, 36–38.

[65] Adrian and Lucas. Journ. Physiol., 1912, xliv, 120.

mal. If the picture they present is a true one for all the synapses in the spinal centers, impulses following each other over a common central path to produce summation must be so timed that succeeding ones shall fall outside the refractory periods following their predecessors and in the supernormal stage of recovery. That the refractory period of any conducting path should be so brief that impulses, separated only by the conduction time of the length of nerve over which the stimulating current might effectively spread, would produce central summation, appears most unlikely.

Sherrington and Sowton[66] have examined specifically the refractory period of the flexion-reflex in the cat by the method of summed contraction, and found it to lie between 1.4σ and 0.7σ in one case, and between 1.1σ and 0.4σ in another. It is not certain from their evidence whether the seat of the refractory period which they are studying is the reflex center or the afferent nerve trunk. Adrian and Lucas[67] have shown that amphibian nerve at 15°C. has an absolute refractory period of about 2σ, and a total refractory period, absolute and relative, of about 12σ. In the experiment of Sherrington and Sowton with the afferent nerve at 33°C. these times were undoubtedly much shortened, perhaps five- or six-fold. But even so, the relative refractory period, during which the propagated disturbances are subnormal, probably persisted more than 1σ. Lucas[68] has shown that such a subnormal disturbance on reaching a "region of decrement," such as he suggests the synapse may be, is apt to be extinguished. In this way a stimulus applied to an afferent nerve during its relative refractory period might fail to augment the reflex muscular contraction, and from its failure we could draw no conclusion as to the refractory period in the synapse. That any part of the central mechanism involved in the spread of reflexes has so brief a refractory period as to admit of summation from the overlapping of arcs in the manner suggested, while conceivable, is so unlikely as to render improbable this explanation of the increments we are considering.

5. Our fifth explanation is suggested by some of the considerations discussed in connection with deformed action current records (p. 203). It will be recalled that some of these gave the appearance of being produced by the passage of two or more impulses instead of one. We discussed theoretical reasons for assuming the possibility that a powerful shock, by maintaining a local excitatory state till the end of the refrac-

[66] Sherrington and Sowton: Loc. cit., 342.
[67] Adrian and Lucas: Loc. cit., 114.
[68] Lucas: Journ. Physiol., 1911, xlii, 72, etc.; ibid., 1913, xlvi, 475.

tory period, might initiate a second propagated disturbance; or even, in the case of shocks of long duration, several disturbances.

The possibility of two or more impulses ensuing in each afferent nerve fiber renders the problem of the central effect entirely different from that of a single impulse. If we follow the suggestion of Adrian and Lucas (vide supra) and regard the central mechanism as a "region of decrement" we may seek an explanation in terms of the summation of propagated disturbances. If we assume that the end branches, synapses or dendrites, involved in the spread of reflexes which occurs only with powerful shocks, have a briefer refractory period than the fibers in the afferent nerve trunk, then we shall have the necessary conditions for central summation. For a second disturbance traversing the afferent fiber in its relative refractory phase might then arrive at the central structure during its supernormal stage of conductivity, and might break through a resistance which blocked the first.[69]

It is premature to assume more than tentatively that the physiology of the central nervous mechanism can be reduced to such simple terms as the impaired conductivity and stages of recovery manifested by a partial block in a nerve trunk. It may well be that a host of other factors must be reckoned with. We cannot be sure that the effect of a second disturbance on the center will be predictable on the basis of the degree of subnormality of the disturbance in the conducting fiber and the degree of supernormality of conduction in the central structure. But it is eminently possible that in some way the central effect of the arrival of two disturbances is different from that of one, and, furthermore, that the effect of two impulses will be modified as they are made to arrive in more or less close succession. In this way it is quite conceivable that an infinite gradation of central effect may be obtainable by the approximation of the second to the first disturbance. Such approximation would result from an increase in the intensity of the local excitatory process which would lead to the initiation of the second disturbance earlier in the relative refractory period.

In support of the view that temporal sequence of propagated disturbances in an afferent nerve may determine the central effect the following observations may be mentioned. In one of those experiments (No. 29) in which myograph tracings were made from the contracting flexor muscle in reflex contraction, we found that with powerful shocks the responses varied strikingly according to whether make shocks or break shocks were employed. The difference is seen in the

[69] See Adrian and Lucas: Loc. cit., 109.

records reproduced in figure 21, showing the responses to two break shocks and two make shocks. They were taken in alternation, the coil distance remaining the same throughout. The break shocks amounted to over 2000 Z units. It will be seen that make and break shocks each consistently produced a characteristic sequence of contractions and relaxations, but that the sequences differ strikingly from each other. With these extremely powerful shocks there can be little doubt that all the afferent fibers were stimulated in each case. The difference in the type of the response must, therefore, relate to some difference between make shocks and break shocks. Such a difference can well be accounted for in accordance with the principles outlined above, on the basis of the known differences of contour between make shocks and break shocks. The local excitatory process may, in each case, have

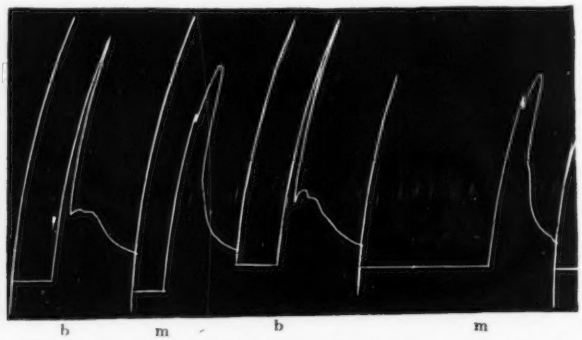


Fig. 21. Reflex contractions of flexor muscle; procedure as in figure 19. Break shocks (b) 2280 Z; make shocks (*m*) at same coil distance (0 mm). Speed of drum 10 mm. per second; ordinate lines show rests. Cu and Hg key.

been maintained long enough to initiate several propagated disturbances. Inasmuch as the rapidity of rise and decline of the electrical disturbances in the two kinds of shock differs, the state of recovery from the refractory period in which the second disturbance was initiated may well have been different in the two cases, resulting in a different timing of the arrival of the series of disturbances in the center. Upon these differences in timing may rest the observed difference in the motor responses.[70]

[70] It is significant in this connection to recall the profound alteration of central effect found by Sherrington and Sowton to result from changing the time relations of the stimulating electric current. Proc. Roy. Soc. B., 1911, lxxxiii, 435.

In another experiment (No. 20) at one coil distance make shocks regularly evoked more reflex activity than break shocks which were rated at 1900 Z, although Martin[71] has shown that by comparison of thresholds make shocks are less intense than break shocks by the same ratio at short coils distances as at long ones; and in our experiments the break shocks must have been several times as powerful by the threshold criterion as the corresponding make shocks.

It is also conceivable that powerful shocks, instead of merely producing a persistent "excitatory state" (ionic concentration), may cause some local damaging effect in the nerve structure, thus providing a somewhat persistent source of excitation, much as is the case with mechanical injury or crushing (cf. p. 217). Such persistence of the source of excitation would give rise to a series of successive impulses, and the considerations just mentioned in connection with a purely electrolytic excitatory process of long duration would apply to this case as well.

Of the five explanations we have considered for the observed increments in the spread of reflexes evoked by shocks of increasing strength which appear to have already become supramaximal, as judged by the action currents derived from the afferent nerve, the third has been definitely excluded by experiment; the fourth appears to be unlikely in view of the extremely short refractory period in the synapse which it would demand; the second we regard as improbable in view of the close parallelism shown to exist between the nerve impulse and its electrical manifestation in all researches which give ground for valid conclusions. The first explanation that (moderate shocks only stimulate the surface layers of fibers and only those contribute appreciably to the recorded action current) we regard as somewhat improbable. This consideration may play a part in explaining the facts, but we doubt if it alone can explain the great increase in central disturbance following the most powerful shocks. The fifth explanation appears to us reasonaby free from objections and on the whole to be the most probable that we have considered.

It seems to us significant that in most cases the marked increments in reflex activity have appeared with the same intensities of shock which have given rise to the deformations of the action current record already discussed in the previous section. Only in a few instances have we found clear increments of reflex effect between two stimuli subsequently shown both to produce maximal (i.e., ungraded) action

[71] Martin: The Measurement of Induction Shocks, 1912, 101.

currents without deformation. It seems probable that whatever causes deformation in the electrical record is the chief cause of increment in the spread of reflexes.

E. Mechanical stimuli

In two experiments mechanical stimuli were applied to nerves in hopes of securing evidence concerning the relation between afferent impulses and central effect under conditions in which non-physiological electrical disturbances were wholly eliminated. To this end a mechanical stimulating device was arranged by which the sharp edge of a steel spring when released struck the nerve lying on a hard rubber plate; three notches were cut in the trigger whereby the force of the blow could be graded, each notch giving a different tension to the spring. Cutting a nerve with scissors gives a mechanical stimulus to every fiber, but the interpretation of the physiological effect is rendered difficult by the fact that a considerable and variable time must elapse between the beginning and the end of transection, enough time to introduce to a marked extent the possibility of summation in common central paths (cf. p. 219). With the spring device the time of stimulation was rendered as nearly simultaneous in all the fibers as could well be done with a mechanical stimulus. In making comparisons between stimuli with this method it was necessary to move the nerve along and bring a fresh portion under the spring each time, for one stroke generally sufficed to impair the nerve considerably. This necessarily limited closely the number of tests which could be made with a given nerve.

In Experiment 15 one set of comparisons of reflex stimuli made with this device at the three strengths available showed definite gradation in the reflex activity. With greater force the response was more vigorous and persistent and the spread of activity to the fore-limb muscles was more pronounced. By way of control, in Experiment 14 galvanometer records were made of the monophasic action currents of a nerve trunk stimulated directly with the mechanical device. These are reproduced in figure 22 in the order in which they were made, together with one produced by a maximal induction shock for comparison. It will be seen that only with the highest notch was the response maximal. *B*, *C*, *D* and *E* all taken with the middle notch show the degree of constancy obtainable with this method. The nerve was shifted to bring the blow in a fresh region after each stimulus in the series except *B* and *D*. Thus *B* and *C* were taken from successive blows on the same spot, and so were *D* and *E*. The impairment following *B* is ob-

vious. It is also evident that the shift in the point of stimulation didn't suffice to make the response in *D* equal to that in *B*. It will be noted that in *D* and *F* second responses appear in the record. This probably indicates that the spring rebounded and struck the nerve a second time. This possibility renders the method unsatisfactory for quantitative comparisons of reflex responses.

The action current of the motor nerve (peroneal) in response to reflex stimulation through the popliteal nerve with the spring device at the lowest notch was recorded monophasically in Experiment 15. The record is produced in figure 23 together with a record of the reflex response to a break shock made a few minutes later without disturbing the apparatus except to substitute the stimulating electrodes for the spring device. It is interesting to note that the general character and

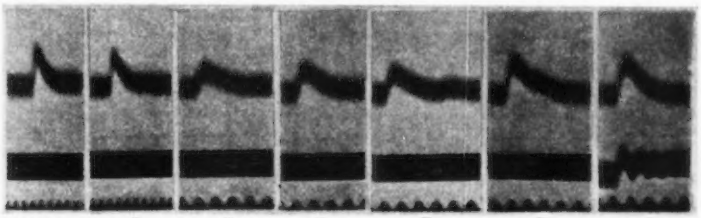


Fig. 22. Responses of peroneal nerve to mechanical stimuli; Experiment 14. Description in text. String D. High-voltage magnet coil with 110-volt current (see legend to figure 15). A, lowest notch; B, C, D and E, middle notch; F, highest notch. Break shock in G for comparison, 59 Z.

time relations of the responses to both reflex and direct stimulation are practically the same with mechanical as with electrical stimuli.

It is evident the spread of reflexes can be induced by a mechanical stimulus so brief that it produces an action current indistinguishable from that evoked by a single induction shock, a fact which argues against the necessity of temporal summation in the causation of such a spread. Beyond this our mechanical stimuli contributed little to the subject under discussion.

III. THEORETICAL IMPLICATIONS OF THE "ALL-OR-NONE" PRINCIPLE

Hitherto we have purposely refrained from accepting Adrian's conclusion that the "all-or-none" principle applies to nerve fiber.[72] We have tentatively assumed it unproved, and proceeded as if gradation

[72] Adrian: Journ. Physiol., 1914, xlvii, 460.

of response were possible in the individual fiber. But Adrian's observations cannot be set aside. They lead, so far as we can see, to no other conclusion than his, namely, that the magnitude of response of nerve fiber, as judged by the ability of the disturbance to propagate itself, is independent of the strength of stimulus. They further show[73] that except in a region of impaired conductivity where the disturbance is actually undergoing a progressive decrement, this magnitude is independent of the previous history of the disturbance; i.e., on emerging from such a region into one of normal conductivity it regains its normal magnitude. If we grant the validity of these conclusions, and if we assume that the magnitude of the electrical response is a valid criterion of the magnitude of the propagated disturbance, then the fact of the limiting maximal value of the action current, which we established experimentally in the first section, becomes an inevitable corollary; for if each fiber responds maximally or not at all then the action current must become maximal when all the fibers are stimulated. Then our preliminary experiments merely show that in a fresh nerve of the size studied all the fibers are stimulated with induction shocks of about 40 or 50 Z units on the Martin scale, unless indeed a considerable number of fibers in the center of the nerve trunk fail to contribute appreciably to the recorded action current.

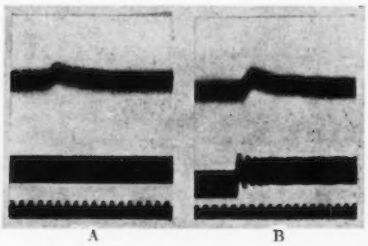


Fig. 23. Experiment 15, reflex responses; description in text. String D. A, mechanical stimulus; B, break shock, 30 Z. Copper point and mercury key.

We propose now to assume the validity of the "all-or-none" law for the nerve fiber, and to consider the consequences of this assumption in interpreting some of the known facts in the physiology of the central nervous system. In the literature are numerous researches in which gradation of magnitude in the nerve impulse is clearly assumed; the results of many strongly suggest that such gradation is possible. In general, the presence of qualitative changes in reflex response correlated with quantitative changes in the strength of afferent stimulation

[73] Adrian: Journ. Physiol., 1912, xlv, 389.

appears hard to reconcile with the "all-or-none" law of the nerve impulse. Why should the involvement of a few more fibers in the nerve trunk change not merely the quantity of reflex response but its entire character? Besides such irradiation of limb reflexes as has been described by Sherrington[74] and those vasomotor phenomena already referred to as studied by Martin and others, there have been notable qualitative differences in the effects of reflex stimulation of different intensities reported by one of us.[75] Whereas prolonged stimulation with shocks of one strength produced augmentation of subsequent reflexes, stronger stimulation caused their depression.

It has been quite customary for writers in this field to assume gradation of the nerve impulse as a matter of course. Thus Sherrington in his exposition of the doctrine of "graded synaptic resistance" as the condition of reflex irradiation, written some years before Adrian's researches, put the case as follows:[76]

> At each synapse there is a neurone-threshold. At each synapse a small quantity of energy, freed in transmission, acts as a releasing force to a fresh store of energy not along a homogeneous train of conducting material as in a nerve-fiber pure and simple, but across a barrier. . . . .

Examining a hypothetical reflex arc *A D* with two synapses, to each of which he assigns a resistance whose numerical value is 2, the afferent, internuncial and motor neurones being designated respectively *A*, *a* and *D*, he goes on to say,

> the resistance along A D need not, on the numerical values assigned to the synapses . . . . . sum to the value 4. Yet it is also clear that the threshold for any whole arc cannot be lower than the highest individual threshold in it. Further, the individual thresholds will tend to sum, for an excitation of neurone A just sufficient to excite neurone *a* is hardly likely to excite *a* sufficiently to overcome the threshold of synapse *a* D.

It is quite clear that in so far as we deal with impulses following each other after intervals sufficient to obviate confusion from the relative refractory period this doctrine is no longer tenable in the light of Adrian's conclusions. The concept of exciting a neurone "sufficiently to overcome" a synaptic threshold is incompatible with the "all-or-none" principle. Furthermore, if a synapse acts, as Adrian and Lucas have

[74] Sherrington: Integrative Action of the Nervous System, 1906, 150–170.

[75] Forbes: Quart. Journ. Exp. Physiol., 1912, v, 178, etc.; This Journal, 1912, xxxi, 116–120.

[76] Sherrington: Op. cit., 155.

suggested,[77] merely as a "region of decrement" comparable to a narcotized stretch of nerve, then the propagated disturbance should, if it were able to pass through the synapse at all, regain its normal size on emerging. If this is the case it renders subject to revision the statement that "the threshold for any whole arc cannot be lower than the highest individual threshold in it." For if a single impulse can pass through the synapses in its path it will induce maximal impulses in every neurone in the chain, and the threshold of the arc must be the threshold of the afferent fiber. Lateral reinforcement from other afferent neurones resulting from the branched arrangement of the central endings might well explain the ability of a volley of impulses in a large number of afferent fibers to excite the central neurones when a smaller number of impulses would fail to do so (cf. p. 219). But the analysis would take a different form from that which assumes gradation of activity in a single fiber.

So far we have dealt with instantaneous stimuli. All the experimental results thus far considered, both in our experiments and in those we have compared with them, have been produced by induction shocks applied to afferent nerves. They all therefore present the same problem discussed in the previous section. We must reconcile the results in each case with the fact that all the effects are evoked by shocks producing full sized impulses in the individual afferent fibers. The differences in the reflex responses must depend either on the number of afferent fibers excited or on some such effect of graded intensity of stimulus as we have considered; i.e., the giving a head start to some impulses with a consequent temporal overlapping in the center (p. 219), or the compounding of excitation in the individual fiber in the manner already analysed (pp. 204 and 221).

But induction shocks applied to nerve trunks constitute a highly artificial laboratory procedure, and it is well to give some consideration to sensory gradation as we know it in normal life. The stimuli by which we are informed of changes in our environment are in a large majority of cases applied not directly to nerve fibers but to special sensory receptors. And in perhaps an equally large majority of cases the stimulus has a duration far exceeding that of the longest induction shock. The importance of these considerations will presently appear.

It is a matter of common experience that we possess the power to distinguish gradations of intensity over a wide range in our ordinary

[77] Adrian and Lucas: Journ. Physiol., 1912, xliv, 120–122.

sensations. The quantitative estimation of this ability constitutes the well known Weber-Fechner law. It is certain that we recognize extensive gradations of intensity, even in the case of minutely localized stimuli, in the sensations of touch, pain and light. The question arises, how can we detect these gradations if the afferent nerve impulses by which the sensations are transmitted to the central nervous system obey the "all-or-none" law? It might be suggested that the degree of sensation depends entirely on the number of afferent fibers excited by the peripheral stimulus. Thus in the case of tactile stimuli gradually increasing pressure on a sharply localized skin point might give evidence of its increase by transmitting adequate pressure to an increasing number of receptors.

Gradation in visual and auditory sensations cannot be explained in this way. In the emmetropic eye the image of a star however bright cannot stimulate more than a single retinal cone at a time. Yet we clearly recognize several different "magnitudes" of stars as differing from each other appreciably in brightness.* If this were due to the successive excitation of more and more nerve fibers in response to increasing activity in a single retinal cone there would have to be as many fibers in the optic nerve connected with each cone as there are separate intensities of illumination which we can distinguish consciously. Howell[78] states that in the fovea at least "each cone connects with a single nerve cell and eventually perhaps with a single optic nerve fiber." Barker[79] states that there are about 1,000,000 cells in the ganglion cell layer of the retina, and about 1,000,000 fibers in each optic nerve. From the size of the retina and the distribution of cones it may justly be estimated that there are at least a million cones in each retina. Thus it is clear that there are not enough optic nerve fibers to admit of sensory gradation by means of graded thresholds in a number of fibers connected with a single cone.

In the case of auditory sensations it is estimated that 4500 resonators must be assumed in order to account for the known discrimination of pitch by the human ear.[80] If we are to assume that our ability to distinguish different intensities in notes of a given pitch depends on the number of nerve fibers thrown into action by the resonator involved, there must be activated by each resonator as many afferent fibers as

*Cf. Graham Brown: Loc. cit., p. 134.

[78] Howell: Text-book of Physiology, third edition, 1910, 350.

[79] Barker: The Nervous System, 1901, 785.

[80] Starling: Principles of Human Physiology, 1912, 576.

there are distinguishable intensities of sensation at that pitch. It is clear that this is not the case, for at a large majority of audible pitches it is easy to demonstrate with a siren that at least ten intensities can be differentiated; careful experimentation would probably reveal much more delicate gradation. Such discrimination would demand at least 40,000 fibers in the acoustic nerve for transmission, and this is far in excess of the actual number, which has been estimated by histologists at about 14,000.[81]

From these elementary facts it follows that even if sensory gradation could conceivably depend, in the case of touch, taste or smell, on the number of afferent fibers excited, no such explanation is tenable for gradation in sight and hearing. Assuming, as we feel we must, the validity of Adrian's conclusion as to the "all-or-none" law, how are we to reconcile it with the obvious sensory gradation unless we assume either that mammalian afferent fibers and amphibian motor fibers, in spite of their similarity in structure and in all objective manifestations of activity, differ fundamentally in the most vital aspects of their physiology, or that the neurofibril and not the fiber is after all the true physiological unit in conduction?

A ready answer to this question is furnished by the researches of Garten, Lucas and Adrian.[82] The various elements in this answer have already been discussed in detail in previous sections of this paper (pp. 205 and 221); it will suffice to summarize them briefly here.

It must be recalled that the "all-or-none" law as applied to excitable tissues does not imply the impossibility of a propagated disturbance being of other than the standard magnitude under any circumstances. Lucas and Adrian[83] have clearly shown that in nerve the relative refractory period, which follows the absolute refractory period, is characterized by lowered excitability and by reduced magnitude of response; a stronger stimulus is required to excite and the resulting response is subnormal. In both respects the tissue returns gradually to normal. When a tissue is stimulated by an electric current a "local excitatory process" is first set up as the direct result of the current, and then a "propagated disturbance" is initiated by the "local excitatory process." The local excitatory process appears to consist in the concentration of certain ions at some limiting membrane within the

[81] See Martin: The Human Body, ninth edition, 1910, 199.

[82] See previous references.

[83] Adrian and Lucas: Loc. cit., 114; Adrian: Journ. Physiol., 1913, xlvi, 389; Lucas: ibid., 1911, xliii, 77.

tissue;[84] this process is subject to gradation. The propagated disturbance appears to resemble an explosion in that it uses up all of the immediately available material involved in its production, and leaves the tissue for a time refractory to stimulation. Excitability depends, first, on the ease with which the local process is set up, and, secondly, on the intensity of the local process required to initiate the propagated disturbance. In the gradual return of excitability to normal following a propagated disturbance we are concerned with the second factor, the nexus between local process and propagated disturbance. If the local excitatory process consists, as seems probable, merely in a concentration of ions at some point, we may safely assume that a constant current or a series of rapidly repeated induction shocks will maintain this process to a certain degree. If either constant current or repeated shocks be sufficiently strong and frequent a continuous stimulus will result. As indicated in an earlier section (p. 204), an excitable tissue, when subjected to constant stimulation, responds rhythmically by virtue of the explosive nature of the response and of some property whereby the tissue is permitted to prepare itself for another response while yet protected by its refractory state from stimulation. The Stannius heart, the classical embodiment of the "all-or-none" law, when tetanized exemplifies this principle, and Garten[85] has shown that similarly a mammalian nerve, stimulated with a constant current of sufficient strength, responds with a series of action currents having a frequency of between 200 and 500 per second at body temperature.

With these facts in mind, we may consider the effect of varying the intensity of stimulation. It is a familiar class room experiment to record the rhythmic contractions of the Stannius heart caused by tetanization with various intensities of induction shock. The gradual return of excitability during the relative refractory period is well illustrated in this way, for with weak stimulation the rhythm of response is slow, while the stronger stimuli the rhythm becomes more rapid; the rate depends upon the stage of recovery at which the particular stimulus becomes adequate.

Adrian[86] has emphasized the same consideration in reference to nerve trunk as follows:

> a strong stimulus will be able to set up a disturbance at a much earlier stage of recovery, when the excitability of the tissue is small. Thus a series of strong stimuli will set up a greater number of disturbances than a series of weak stimuli.

[84] See Hill: Journ. Physiol., 1910, xl, 190; Lucas: ibid., xl, 225.

[85] Garten: Loc. cit., 557.

[86] Adrian: Journ. Physiol., 1913, xlvi, 385-386.

In applying this principle to sensory gradation, we are handicapped by knowing little of the laws governing excitation in receptors. We may suppose that their responses to graded stimuli are graded. It may also be that when adequately stimulated they maintain what amounts, as far as the nerve fibers are concerned, to a constant source of stimulation analogous either to a constant current or to a series of stimuli so rapid that their relation to the refractory period in nerve is comparable to that of the tetanizing stimulus to the refractory period of the heart. In absence of more definite knowledge we may fairly assume that this is the case. The fact that sound waves of much lower frequency produce the sensation of a musical note need not interfere with such an assumption, for it is quite conceivable that the resonator serves as a rhythm transformer, passing on to the acoustic nerve endings a virtually constant, or at least a far more rapidly rhythmic stimulus than the sound waves which agitate it. It is not unlikely that after-discharge is a characteristic property of all receptors as it so manifestly is of those concerned with sight. If this were so, then the briefest sensory stimulus, such as a flash of lightning, would be transmuted into a stimulus whose duration would far outlast the refractory period of the nerve fiber to which it was imparted by the receptor.

If the stimulus transmitted by the receptor to the nerve fiber possesses these qualities, gradation, continuity (or virtual continuity) and duration beyond the refractory phase of the nerve, then a basis exists for infinitely graded perception of the strength of sensory stimulation. For as with the Stannius heart, so with the nerve unlimited gradations of frequency in the rhythm of response are possible, each corresponding to a different strength of stimulation. We see no objection to the hypothesis that the brain interprets as evidence of the intensity of peripheral stimulation the rhythm of the nerve impulses by which the sensation is mediated. On this view increasing intensity of sensation would have for its basis increasing frequency of impulses in the afferent nerve fibers.

IV. SUMMARY

1. When a mammalian nerve trunk, such as the sciatic or one of its major branches (popliteal or peroneal) in the cat, is stimulated with single induction shocks of graded intensity, and the resulting action currents are recorded monophasically with the string galvanometer, the magnitude of the electrical response normally increases with in-

creasing stimuli until the latter have reached a value in the neighborhood of 40 Z units; with further increase in strength of stimulus no further increase in response occurs so long as this retains the form typical of a simple action current record, in short there is a limiting maximal value to the action current. When the increase in the strength of induction shock is carried far enough (usually about 200 z in round numbers) the electrical responses no longer appear as simple curves, but show deformation which becomes increasingly marked as the strength of shock is further increased.

If the stimuli be applied to a portion of the nerve which has suffered injury or impairment, the limiting maximal value may be only gradually approached by the response and never quite reached with any stimulus yielding records without deformation, indeed under these circumstances the maximum response obtainable may be considerably smaller than that which results from stimulation in a fresh uninjured portion of the nerve. The failure of such cases to show a definite maximal limit is taken to indicate greatly lowered excitability in many of the nerve fibers, and not to invalidate the general conclusion.

If the action current is a true measure of physiological activity a limiting maximal value of response must of course occur, according to the "all-or-none" principle, as soon as all the fibers are stimulated. In view of this the demonstration of a limiting maximal value of the action current supports the theory that the latter is a true measure of the nerve impulse and in the individual fiber obeys the "all-or-none" law.

2. The causes of deformation in the action current record have been investigated by differential elimination of as many possible contributing factors as we found subject to control.

It is pointed out that the recording instrument need not lie in the path of any part of the current flowing between the stimulating electrodes in order that an electrical disturbance coming directly from the latter shall appear in the record.

Some of the deformed action current records are too complex to be interpreted by consideration of known physical factors. Make shocks in general cause more striking deformation than break shocks.

It is suggested that some at least of the deformations may result from the initiation of a second and even a third propagated disturbance as a result of the "local excitatory process" being maintained with sufficient intensity till after the refractory period following the first. (Cf. Garten.)

3. In examining the question of graded correlation between reflex response and stimulus, in the decerebrate animal, our most significant results have come from inspection. We find that with single shocks the intensity of response in the muscles involved in the flexion reflex and the extensiveness of the spread of reflex activity to remote muscle groups both exhibit striking increments correlated not only with those increments of stimulus which can be shown to produce corresponding increments in the action current of the afferent nerve, but also with increments of stimulus far above the demonstrated maximal value. If we may assume that the electrical response is a true criterion and measure of physiological activity and that activity in all the fibers in the nerve can contribute appreciably to the action current as recorded in the galvanometer, then this long continued series of increments presents an interesting paradox.

Of the conceivable explanations of this phenomenon that have occurred to us we incline to regard as the most probable that a second propagated disturbance, or even a series of them, may be evoked in each afferent nerve fiber by a single shock if sufficiently strong. The arrival of successive impulses in the center may produce a wholly different effect from that of a single impulse, and in the graded rapidity of succession between the impulses there might be the basis of an unlimited gradation in the character or intensity of the reflex response.

4. Mechanical stimuli have been applied to nerve trunks, and action currents have been recorded both from the stimulated nerve and from the motor nerve involved in a reflex response to the stimulus.

5. Our experiments call to mind an apparent incompatibility of the "all-or-none" law (established by Adrian for frog's motor nerve, and presumably applying also to vertebrate nerve fibers in general, both efferent and afferent) and the doctrine of "graded synaptic resistance" which has been used to explain the familiar spread of reflex activity in response to stimuli of increasing strength. The need of revision of some hitherto accepted principles is apparent.

In particular the "all-or-none" law appears incompatible with the delicate gradation of sensory discrimination between different intensities of peripheral stimulation, which is apparent in conscious life. It is shown that in the case of sight and hearing such discrimination cannot be explained by the assumption that an additional afferent nerve fiber is excited at each perceptible increase in intensity of sensory stimulation. It is therefore suggested that inasmuch as excitable tissues respond rhythmically to constant stimulation in consequence of the re-

fractory period, the rhythm of afferent nerve impulses may afford a basis for sensory discrimination of intensity of peripheral stimulation. The work of Lucas and Adrian shows that the recovery of excitability in nerve following its refractory period is such that a sustained stimulus of great intensity would produce a more rapidly rhythmic response than one of less intensity. If the sensory receptors provide to the afferent fibers sustained stimuli of graded intensity, the brain may be apprised of the intensity of the peripheral stimulus by the frequency with which the impulses come to the centers over the afferent nerve fibers.

FURTHER EVIDENCE OF A VASOTONIC AND A VASOREFLEX MECHANISM

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I

In the first research on this subject[1] evidence was presented that the general arterial tonus and the vasomotor reflexes are not controlled by the same nerve center.

A second research established a vasotonic and a vasoreflex center.[2] The method consisted in applying a single reagent to the vasomotor center hitherto believed to control both the vasotonic and the vasoreflex function. If the center were indeed single, the changes produced by the reagent in the two functions should have been in the same direction. When the reagent increased the reflexes, it should also have increased the tonus. If both tonus and reflexes are the results of the energy of one and the same nerve cell, both functions should have been augmented or depressed as the energy of the cell was augmented or depressed. But curare, the agent employed in those experiments, did not produce this reaction. Only one of the functions was altered. Curare left the tonus unchanged while the depressor and the sciatic vasomotor reflexes were more than doubled. Curare thus separated the vasoreflex from the vasotonic function.

The present investigation confirms that demonstration. It will appear that certain doses of alcohol leave the tonus unchanged while completely suspending the vasomotor reflexes.

[1] W. T. Porter. This journal, 1910, xxvii, 276.
[2] Ibid., 1915, xxxvi, 418.

II

In figure 1 is shown a typical experiment. On October 22, 1915,[3] a membrane manometer was connected to the carotid artery of an etherized rabbit. In the right jugular vein was placed a cannula to which a glass syringe was attached. The left depressor nerve was prepared. On stimulating this nerve with maximal induction currents the normal depressor reflex was obtained, as shown to the left in figure 1; the blood pressure fell from 110 to 75 mm. Hg. Three cubic centimeters of 75 per cent alcohol were now injected into the vein. Three minutes

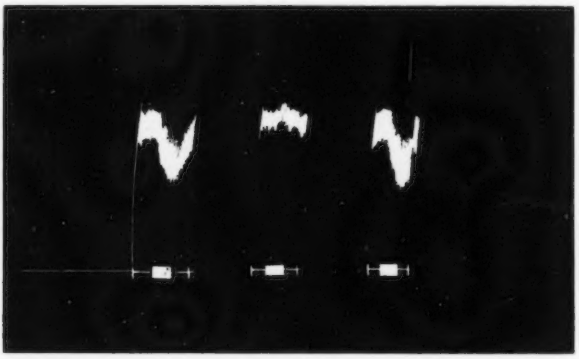

Fig. 1. The effect of alcohol on the arterial tonus and the depressor reflex in the rabbit. The reflex disappears, but soon returns. The tonus remains throughout substantially unchanged.

later, the depressor was again stimulated, but the reflex had disappeared, though the tonus was substantially unchanged. After about five minutes, a third stimulation caused the blood pressure to fall from 108 to 70 mm. Hg. The reflex had returned. The tonus was still practically at the same level.[4]

[3] The phenomenon was first observed in November, 1914. A large number of records were obtained in November, December and January. The entire investigation was repeated in March, 1915, and again in October, 1915. In all three series the results were the same.

[4] It is easy to obtain a lessening of the reflex, but the greatest care must be used if a perfect result is desired. The least mismanagement of the anaesthetic, or the least error in the amount of alcohol, the speed of the injection, or the interval between injection and stimulation will defeat the observation.

Alcohol therefore has an effect opposite to that of curare. With curare, the tonus remains unchanged but the reflex doubles; with alcohol, the tonus remains unchanged but the reflex disappears. Neither phenomenon would be possible, if the vasotonic and the vasoreflex mechanism were identical.